Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	582	acetylglucosaminyltransferase$ or acetylglucosamin$ transferase$ or GlcNac adj (t$1 or transferase$)	US-PGPUB; USPAT	ADJ	OFF	2004/06/17 14:48
L2	74	1 near5 (gene$1 or sequence$1)	US-PGPUB; USPAT	OR	OFF	2004/06/17 15:05
L3	36	1 near2 "3"	US-PGPUB; USPAT	OR	OFF	2004/06/17 14:50
L4	13	2 and 3	US-PGPUB; USPAT	OR	OFF	2004/06/17 15:05
L5	70	1 near5 human	US-PGPUB; USPAT	OR	OFF	2004/06/17 15:05
L6	7	5 and 3	US-PGPUB; USPAT	OR	OFF	2004/06/17 15:05

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	582	acetylglucosaminyltransferase$ or acetylglucosamin$ transferase$ or GlcNac adj (t$1 or transferase$)	US-PGPUB; USPAT	ADJ	OFF	2004/06/17 14:48
L2	74	1 near5 (gene$1 or sequence$1)	US-PGPUB; USPAT	OR	OFF	2004/06/17 14:49
L3	36	1 near2 "3"	US-PGPUB; USPAT	OR	OFF	2004/06/17 14:50
L4	13	2 and 3	US-PGPUB; USPAT	OR	OFF	2004/06/17 14:51

PGPUB-DOCUMENT-NUMBER: 20040018590

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018590 A1

TITLE: Combinatorial DNA library for producing modified N-glycans in lower eukaryotes

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gerngross, Tillman U.	Hanover	NH	US	
Wildt, Stefan	Lebanon	NH	US	
Choi, Byung-Kwon	Norwich	VT	US	
Nett, Juergen Hermann	Grantham	NH	US	
Bobrowicz, Piotr	White River Junction	VT	US	
Hamilton, Stephen R.	Enfield	NH	US	
Davidson, Robert C.	Enfield	NH	US	

APPL-NO: 10/ 371877

DATE FILED: February 20, 2003

RELATED-US-APPL-DATA:

child 10371877 A1 20030220

parent continuation-in-part-of 09892591 20010627 US PENDING

non-provisional-of-provisional 60214358 20000628 US

non-provisional-of-provisional 60215638 20000630 US

non-provisional-of-provisional 60279997 20010330 US

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 530/395 , 536/23.2 , 536/53

ABSTRACT:

The present invention relates to eukaryotic host cells having modified oligosaccharides which may be modified further by heterologous expression of a set of glycosyltransferases, sugar transporters and mannosidases to become host-strains for the production of mammalian, e.g., human therapeutic glycoproteins. The invention provides nucleic acid molecules and combinatorial libraries which can be used to successfully target and express mammalian enzymatic activities such as those involved in glycosylation to intracellular compartments in a eukaryotic host cell. The process provides an engineered host cell which can be used to express and target any desirable gene(s) involved in glycosylation. Host cells with modified oligosaccharides are created or selected. N-glycans made in the engineered host cells have a Man.sub.5GlcNAc.sub.2 core structure which may then be modified further by heterologous expression of one or more enzymes, e.g., glycosyltransferases, sugar transporters and mannosidases, to yield human-like glycoproteins. For the production of therapeutic proteins, this method may be adapted to engineer cell lines in which any desired glycosylation structure may be obtained.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/892,591, filed Jun. 27, 2001, in which priority is claimed to U.S. Provisional Application Serial No. 60/214,358, filed Jun. 28, 2000, U.S. Provisional Application No. 60/215,638, filed Jun. 30, 2000, and U.S. Provisional Application No. 60/279,997, filed Mar. 30, 2001; each of which is incorporated herein by reference in its entirety.

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Detail Description Paragraph - DETX (187):
[0240] Examples of modifications to glycosylation which can be affected using a method according to this embodiment of the invention are: (1) engineering a eukaryotic host cell to trim mannose residues from Man.sub.8GlcNAc.sub.2 to yield a Man.sub.5GlcNAc.sub.2 N-glycan; (2) engineering eukaryotic host cell to add an N-acetylglucosamine (GlcNAc) residue to Man.sub.5GlcNAc.sub.2 by action of GlcNAc transferase I; (3) engineering a eukaryotic host cell to functionally express an enzyme such as an N-acetylglucosaminyl Transferase (GnTI, GnTII, GnTIII, GnTIV, GnTV, GnTVI), mannosidase II, fucosyltransferase (FT), galactosyl tranferase (GalT) or a sialyltransferase (ST).

Detail Description Paragraph - DETX (290):
[0312] GlcNAc Transferase I activity is required for the maturation of complex and hybrid N-glycans (U.S. Pat. No. 5,834,251). Man.sub.5GlcNAc.sub.2 may only be trimmed by mannosidase II, a necessary step in the formation of human glycoforms, after the addition of N-acetylglucosamine to the terminal .alpha.-1,3 mannose residue of the trimannose stem by GlcNAc Transferase I (Schachter, 1991 Glycobiology 1(5):453-461). Accordingly, a combinatorial DNA library was prepared including DNA fragments encoding suitably targeted catalytic domains of GlcNAc Transferase I genes from C. elegans and Homo sapiens; and localization sequences from GLS, MNS, SEC, MNN9, VAN1, ANP1, HOC1, MNN10, MNN11, MNT1, KTR1, KTR2, MNN2, MNN5, YUR1, MNN1, and MNN6 from S. cerevisiae and P. pastoris putative .alpha.-1,2-mannosyltransferases based on the homology from S. cerevisiae: D2, D9 and J3, which are KTR homologs. Table 10 includes but does not limit targeting peptide sequences such as SEC and OCH1, from P. pastoris and K. lactis GnTI, (See Table 6 and Table 10)

Detail Description Paragraph - DETX (292):
[0314] A portion of the gene encoding human N-acetylglucosaminyl Transferase I (MGATI, Accession# NM002406), lacking the first 154 bp, was amplified by PCR using oligonucleotides 5'-TGGCAGGCGCGCCTCAGTCAGCGCTCTCG-- 3' (SEQ ID NO:32) and 5'-AGGTTAATTA AGTGCTAATTCCAGCTAGG-3' (SEQ ID NO:33) and vector pHG4.5 (ATCC# 79003) as template. The resulting PCR product was cloned into pCR2.1-TOPO and the correct sequence was confirmed. Following digestion with AscI and PacI the truncated GnTI was inserted into plasmid pJN346 to create pNA. After digestion of pJN271 with NotI and AscI, the 120 bp insert was ligated into pNA to generate an in-frame fusion of the MNN9 transmembrane domain with the GnTI, creating pNA 15.

PGPUB-DOCUMENT-NUMBER: 20030180778

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030180778 A1

TITLE: UDP-N-acetylglucosamine: galactose-beta1,3-N-acetylgalactosamine-alpha-R/ (GlcNAc to GalNAc) beta1,6-N-acetylglucosaminyltransferase, C2GnT3

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schwientek, Tilo	Bronshoj		DK	
Clausen, Henrik	Holte		DK	

APPL-NO: 10/ 388307

DATE FILED: March 13, 2003

RELATED-US-APPL-DATA:

child 10388307 A1 20030313

parent continuation-of 09645192 20000824 US PENDING

non-provisional-of-provisional 60150488 19990824 US

US-CL-CURRENT: 435/6, 435/193 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

A novel gene defining a novel human UDP-GlcNAc: Gal.beta.1-3GalNAc.alpha. .beta.1,6GlcNAc-transferase, termed C2GnT3, with unique enzymatic properties is disclosed. The enzymatic activity of C2GnT3 is shown to be distinct from that of previously identified enzymes of this gene family. The invention discloses isolated DNA molecules and DNA constructs encoding C2GnT3 and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting C2GnT3 activity, as well as cloning and expression vectors including such DNA, cells transfected with the vectors, and recombinant methods for providing C2GnT3. The enzyme C2GnT3 and C2GnT3-active derivatives thereof are disclosed, in particular soluble derivatives comprising the catalytically active domain of C2GnT3. Further, the invention discloses methods of obtaining 1,6-N-acetylglucosaminyl glycosylated saccharides, glycopeptides or glycoproteins by use of an enzymically active C2GnT3 protein or fusion protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active C2GnT3 protein as an expression system for recombinant production of such glycopeptides or glycoproteins. Methods are disclosed for the identification of agents with the ability to inhibit or stimulate the biological activity of C2GnT3. Furthermore, methods of using C2GnT3 in the structure-based design of inhibitors or stimulators thereof are also disclosed in the invention. Also a method for the identification of DNA sequence variations in the C2GnT3 gene by isolating DNA from a patient, amplifying C2GnT3-coding exons by PCR, and detecting the presence of DNA sequence variation, are disclosed.

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Summary of Invention Paragraph - BSTX (10):
[0008] Consequently, there exists a need in the art for detecting as yet unidentified UDP-N-acetylglucosamine: Galactose-.beta.1,3-N-acetylgalacto-samine-.alpha.-R (GlcNAc to GalNAc) .beta.1-6 N-acetylglucosaminyltransfer-ases and identifying the primary structures of the genes encoding such enzymes. The present invention meets this need, and further presents other related advantages.

Summary of Invention Paragraph - BSTX (12):
[0009] The present invention provides isolated nucleic acids encoding human UDP-N-acetylglucosamine: N-acetylgalactosamine .beta.1,6 N-acetylglucosaminyltransferase 3 (C2GnT3), including cDNA and genomic DNA. C2GnT3 has acceptor substrate specificities comparable to C2GnT1 (14). The complete nucleotide sequence encoding C2GnT3 is set forth in SEQ ID NO:1 and in FIG. 1.

Detail Description Paragraph - DETX (17):
[0048] The present invention provides the isolated DNA molecules, including genomic DNA and cDNA, encoding the UDP-N-acetylglucosamine: N-acetylgalactosamine .beta.1,6 N-acetylglucosaminyl-transferase 3 (C2GnT3).

Detail Description Paragraph - DETX (185):
[0210] 22. Bierhuizen, M. F., Maemura, K., Kudo, S., and Fukuda, M. Genomic organization of core 2 and I branching beta-1,6-N-acetylglucosami-nyltransferases. Implication for evolution of the beta-1,6-N-acetylglucosa-minyltransferase gene family. Glycobiology 5: 417-425, 1995.

PGPUB-DOCUMENT-NUMBER: 20030054525

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054525 A1

TITLE: UDP-N-acetylglucosamine: galactose-beta1,3-N-acetylgalactosamine-alpha-R / (GlcNAc to GalNAc) beta1,6-N-acetylglucosaminyltransferase, C2GnT3

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schwientek, Tilo	Bronshoj		DK	
Clausen, Henrik	Holte		DK	

APPL-NO: 10/ 084406

DATE FILED: February 25, 2002

RELATED-US-APPL-DATA:

child 10084406 A1 20020225

parent division-of 09645192 20000824 US PENDING

non-provisional-of-provisional 60150488 19990824 US

US-CL-CURRENT: 435/193, 435/252.3 , 435/254.2 , 435/320.1 , 435/325 , 435/348 , 435/69.1 , 536/23.2

ABSTRACT:

A novel gene defining a novel human UDP-GlcNAc: Gal.beta.1-3GalNAc.alpha. .beta.1,6GlcNAc-transferase, termed C2GnT3, with unique enzymatic properties is disclosed. The enzymatic activity of C2GnT3 is shown to be distinct from that of previously identified enzymes of this gene family. The invention discloses isolated DNA molecules and DNA constructs encoding C2GnT3 and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting C2GnT3 activity, as well as cloning and expression vectors including such DNA, cells transfected with the vectors, and recombinant methods for providing C2GnT3. The enzyme C2GnT3 and C2GnT3 -active derivatives thereof are disclosed, in particular soluble derivatives comprising the catalytically active domain of C2GnT3. Further, the invention discloses methods of obtaining 1,6-N-acetylglucosaminyl glycosylated saccharides, glycopeptides or glycoproteins by use of an enzymically active C2GnT3 protein or fusion protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active C2GnT3 protein as an expression system for recombinant production of such glycopeptides or glycoproteins. Methods are disclosed for the identification of agents with the ability to inhibit or stimulate the biological activity of C2GnT3. Furthermore, methods of using C2GnT3 in the structure-based design of inhibitors or stimulators thereof are also disclosed in the invention. Also a method for the identification of DNA sequence variations in the C2GnT3 gene by isolating DNA from a patient, amplifying C2GnT3-coding exons by PCR, and detecting the presence of DNA sequence variation, are disclosed.

[0001] This application is a divisional of U.S. Ser. No. 09/645,192, filed Aug. 24, 2000, which claims priority to U.S. Ser. No. 60/150,488, filed Aug. 24, 1999. Each of these prior applications is incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (10):
[0009] Consequently, there exists a need in the art for detecting as yet unidentified UDP-N-acetylglucosamine: Galactose-.beta.1,3-N-acetylgalacto-samine-.alpha.-R (GlcNAc to GalNAc) .beta.1-6 N-acetylglucosaminyltransfer-ases and identifying the primary structures of the genes encoding such enzymes. The present invention meets this need, and further presents other related advantages.

Summary of Invention Paragraph - BSTX (12):
[0010] The present invention provides isolated nucleic acids encoding human UDP-N-acetylglucosamine: N-acetylgalactosamine .beta.1,6 N-acetylglucosaminyltransferase 3 (C2GnT3), including cDNA and genomic DNA. C2GnT3 has acceptor substrate specificities comparable to C2GnT1 (14). The complete nucleotide sequence encoding C2GnT3 is set forth in SEQ ID NO: 1 and in FIG. 1.

Detail Description Paragraph - DETX (17):
[0047] The present invention provides the isolated DNA molecules, including genomic DNA and cDNA, encoding the UDP-N-acetylglucosamine: N-acetylgalactosamine .beta.1,6 N-acetylglucosaminyltransferase 3 (C2GnT3).

Detail Description Paragraph - DETX (182):
[0186] 22. Bierhuizen, M. F., Maemura, K., Kudo, S., and Fukuda, M. Genomic organization of core 2 and I branching beta-1,6-N-acetylglucosaminyltransferases. Implication for evolution of the beta-1,6-N-acetylglucosaminyltransferase gene family. Glycobiology 5: 417-425, 1995.

PGPUB-DOCUMENT-NUMBER: 20020137134

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137134 A1

TITLE: Methods for producing modified glycoproteins

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gerngross, Tillman U.	Hanover	NH	US	

APPL-NO: 09/ 892591

DATE FILED: June 27, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60214358 20000628 US

non-provisional-of-provisional 60215638 20000630 US

non-provisional-of-provisional 60279997 20010330 US

US-CL-CURRENT: 435/69.1, 435/200 , 435/254.23 , 435/320.1 , 530/395

ABSTRACT:

Cell lines having genetically modified glycosylation pathways that allow them to carry out a sequence of enzymatic reactions, which mimic the processing of glycoproteins in humans, have been developed. Recombinant proteins expressed in these engineered hosts yield glycoproteins more similar, if not substantially identical, to their human counterparts. Thelower eukaryotes, which ordinarily produce high-mannose containing N-glycans, including unicellular and multicellular fungi are modified to produce N-glycans such as Man.sub.5GlcNAc.sub.2 or other structures along human glycosylation pathways. This is achieved using a combination of engineering and/or selection of strains which: do not express certain enzymes which create the undesirable complex structures characteristic of the fungal glycoproteins, which express exogenous enzymes selected either to have optimal activity under the conditions present in the fungi where activity is desired, or which are targeted to an organelle where optimal activity is achieved, and combinations thereof wherein the genetically engineered eukaryote expresses multiple exogenous enzymes required to produce "human-like" glycoproteins.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] Priority is claimed to U.S. Provisional Application Serial No. 60/214,358, filed on Jun. 28, 2000, U.S. Provisional Application Serial No. 60/215,638, filed on Jun. 30, 2000, and U.S. Provisional Application Serial No. 60/279,997, filed on Mar. 30, 2001.

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Detail Description Paragraph - DETX (37):

[0082] Examples of modifications to glycosylation which can be effected using method are: (1) engineering an eukaryotic microorganism to trim mannose residues from Man.sub.8GlcNAc.sub.2 to yield Man.sub.5GlcNAc.sub.2 as a protein N-glycan; (2) engineering an eukaryotic microorganism to add an N-acetylglucosamine (GlcNAc) residue to Man.sub.5GlcNAc.sub.2 by action of GlcNAc transferase I; (3) engineering an eukaryotic microorganism to functionally express an enzyme such as an N-acetylglucosamine transferase (GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI), mannosidase II, fucosyltransferase, galactosyl tranferase (GalT) or sialyltransferases (ST).

Detail Description Paragraph - DETX (60):

[0102] GlcNAc Transferase I activity is required for the maturation of complex N-glycans. Man.sub.5GlcNAc.sub.2 may only be trimmed by mannosidase II, a necessary step in the formation of human glycoforms, after the addition of GlcNAc to the terminal .alpha.-1,3 mannose residue by GlcNAc Transferase I (Schachter, 1991 Glycobiology 1(5):453-461). Accordingly a library is prepared including DNA fragments encoding suitably targeted GlcNAc Transferase I genes. The host organism is a strain, e.g. a yeast, that is deficient in hypermannosylation (e.g. an OCH1 mutant), provides the substrate UDP-GlcNAc in the Golgi and/or ER, and provides N-glycans of the structure Man.sub.5GlcNAc.sub.2 in the Golgi and/or ER. After transformation of the host with the DNA library, the transformants are screened for those having the highest concentration of terminal GlcNAc on the cell surface, or alternatively secrete the protein having the highest terminal GlcNAc content. Such a screen is performed using a visual method (e.g. a staining procedure), a specific terminal GlcNAc binding antibody, or a lectin. Alternatively the desired transformants exhibit reduced binding of certain lectins specific for terminal mannose residues.

PGPUB-DOCUMENT-NUMBER: 20020081656

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081656 A1

TITLE: UDP-N-acetylglucosamine: Galactose-beta1,3-N-acetylgalactosamine-alpha-R / N-acetylglucosamine-beta1,3,-N-acetylgalactosamine-alpha-R (GlcNAc to GalNAc) beta1,6-N-acetylglucosaminyltransferase, C2/4GnT

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Clausen, Henrik	Holte		DK	
Schwientek, Tilo	Bronshoj		DK	

APPL-NO: 09/ 874390

DATE FILED: June 4, 2001

RELATED-US-APPL-DATA:

child 09874390 A1 20010604

parent continuation-of PCT/DK99/00677 19991203 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 1998 01605	1998DK-PA 1998 01605	December 4, 1998

US-CL-CURRENT: 435/69.1, 435/193 , 435/320.1 , 435/325 , 435/6 , 536/23.2

ABSTRACT:

A novel gene defining a novel human UDP-GlcNAc: Gal/Gl cNAc.beta. 1-3GalNAc a.beta.1, 6GlcNAc-transferase, termed C2/4GnT, with unique enzymatic properties is disclosed. The enzymatic activity of C2/4GnT is shown to be distinct from that of previously identified enzymes of this gene family. The invention discloses isolated DNA molecules and DNA constructs encoding C2/4GnT and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting C2/4GnT activity, as well as cloning and expression vectors including such DNA, cells transfected with the vectors, and recombinant methods for providing C2/4GnT. The enzyme C2/4GnT and C2/4GnT-active derivatives thereof are disclosed, in particular soluble derivatives comprising the catalytically active domain of C2/4GnT. Further, the invention discloses methods of obtaining 1,6-N-acetyl glucosaminyl glycosylated saccharides, glycopeptides or glycoproteins by use of an enzymically active C2/4GnT protein or fusion protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active C2/4GnT protein as an expression system for recombinant production of such glycopeptides or glycoproteins. Also a method for the identification for the identification of DNA sequence variations in the C2/4GnT gene by isolating DNA from a patient, amplifying C2/4GnT-coding exons by PCR, and detecting the presence of DNA sequence variation are disclosed.

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Summary of Invention Paragraph - BSTX (8):
[0006] Consequently, there exists a need in the art for UDP-N-Acetylglucosamine: Galactose-.beta.1,3-N-Acetylgalactosamine-.alpha-.-R/N-Acetylglucosamine-.beta.1, 3-N-Acetyl-galactosamine-.alpha.-R (GlcNAc to GalNAc) .beta.1-6 N-Acetylglucosaminyltransferase and the primary structure of the gene encoding these enzyme. The present invention meets this need, and further presents other related advantages.

Brief Description of Drawings Paragraph - DRTX (2):
[0013] FIG. 1 depicts the biosynthetic pathways of mucin-type O-glycan core structures. The abbreviations used are GalNAc-T: polypeptide .alpha.GalNAc-transferase; ST6GalNAcI: mucin .alpha.2,6 sialyltransferase; C 1.beta.3Gal-T: core I .beta.1,3 galactosyl-transferase; C2GnT: core 2 .beta.1,6 GlcNAc-transferase; C2/4GnT- core2/core 4 .beta.1,6 GlcNAc-transferase; C3GnT: core 3 .beta.1,3 GlcNAc-transferase; ST3GalI: mucin .alpha.2,3 sialyltransferase; .beta.4Gal-T: .beta.1,4 galactosyltransferase; .beta.3Gal-T: .beta.1,3 galactosyl-transferase; .beta.3GnT: elongation .beta.1,3 GlcNAc-transferase.

Detail Description Paragraph - DETX (91):
[0104] 13. Bierhuizen, M. F., Maemura, K., Kudo, S., and Fukuda, M. Genomic organization of core 2 and I branching beta-1,6-N-acetylglucosaminyltransferases. Implication for evolution of the beta-1,6-N-acetylglucosaminyltransferase gene family. Glycobiology, 5:417425, 1995.

US-PAT-NO: 6635461

DOCUMENT-IDENTIFIER: US 6635461 B1

TITLE: UDP-N-acetylglucosamine: galactose-.beta.1, 3-N-acetylgalactosamine-.alpha.-R/(GlcNAc to GalNAc) .beta.1,6-N-acetylglucosaminyltransferase, C2GnT3

DATE-ISSUED: October 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schwientek; Tilo	Br.o slashed.nsh.o	N/A	N/A	DK
Clausen; Henrik	slashed.j Holte	N/A	N/A	DK

APPL-NO: 09/ 645192

DATE FILED: August 24, 2000

PARENT-CASE:

This application claims priority to U.S. Provisional application 60/150,488 filed Aug. 24, 1999 now abandoned.

US-CL-CURRENT: 435/193, 435/252.3 , 435/320.1 , 435/325 , 435/6 , 536/23.2

ABSTRACT:

A novel gene defining a novel human UDP-GlcNAc: Gal.beta.1-3 GalNAc.alpha. .beta.1,6GlcNAc-transferase, termed C2GnT3, with unique enzymatic properties is disclosed. The enzymatic activity of C2GnT3 is shown to be distinct from that of previously identified enzymes of this gene family. The invention discloses isolated DNA molecules and DNA constructs encoding C2GnT3 and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting C2GnT3 activity, as well as cloning and expression vectors including such DNA, cells transfected with the vectors, and recombinant methods for providing C2GnT3. The enzyme C2GnT3 and C2GnT3-active derivatives thereof are disclosed, in particular soluble derivatives comprising the catalytically active domain of C2GnT3. Further, the invention discloses methods of obtaining 1,6-N-acetylglucosaminyl glycosylated saccharides, glycopeptides or glycoproteins by use of an enzymically active C2GnT3 protein or fusion protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active C2GnT3 protein as an expression system for recombinant production of such glycopeptides or glycoproteins. Methods are disclosed for the identification of agents with the ability to inhibit or stimulate the biological activity of C2GnT3. Furthermore, methods of using C2GnT3 in the structure-based design of inhibitors or stimulators thereof are also disclosed in the invention. Also a method for the identification of DNA sequence variations in the C2GnT3 gene by isolating DNA from a patient, amplifying C2GnT3-coding exons by PCR, and detecting the presence of DNA sequence variation, are disclosed.

24 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (10):
Consequently, there exists a need in the art for detecting as yet unidentified UDP-N-acetylglucosamine: Galactose-.beta.1,3-N-acetylgalactosamine-.alpha.-R (GlcNAc to GalNAc) .beta.1-6 N-acetylglucosaminyltransferases and identifying the primary structures of the genes encoding such enzymes. The present invention meets this need, and further presents other related advantages.

Brief Summary Text - BSTX (12):
The present invention provides isolated nucleic acids encoding human UDP-N-acetylglucosamine: N-acetylgalactosamine .beta.1,6 N-acetylglucosaminyltransferase 3 (C2GnT3), including cDNA and genomic DNA. C2GnT3 has acceptor substrate specificities comparable to C2GnT1 (14). The complete nucleotide sequence encoding C2GnT3 is set forth in SEQ ID NO: 1 and in FIG. 1.

Detailed Description Text - DETX (17):
The present invention provides the isolated DNA molecules, including genomic DNA and cDNA, encoding the UDP-N-acetylglucosamine: N-acetylgalactosamine .beta.1,6 N-acetylglucosaminyl-transferase 3 (C2GnT3).

Detailed Description Text - DETX (191):
22. Bierhuizen; M. F., Maemura, K., Kudo, S., and Fukuda, M. Genomic organization of core 2 and I branching beta-1,6-N-acetylglucosaminyltransferases. Implication for evolution of the beta-1,6-N-acetylglucosaminyltransferase gene family. Glycobiology 5: 417-425, 1995.

US-PAT-NO: 6420149

DOCUMENT-IDENTIFIER: US 6420149 B1
See image for Certificate of Correction

TITLE: Polypeptides

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fukuda; Minoru	San Diego	CA	N/A	N/A
Sasaki; Katsutoshi	Sagamihara	N/A	N/A	JP
Miura; Kazumi	Fujisawa	N/A	N/A	JP
Nakagawa; Satoshi	Machida	N/A	N/A	JP
Nishi; Tatsunari	Higashimine-machi	N/A	N/A	JP
Sekine; Susumu	Yokohama	N/A	N/A	JP

APPL-NO: 09/ 182450

DATE FILED: October 30, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	9-300715	October 31, 1997

US-CL-CURRENT: 435/193, 435/183 , 435/252.3 , 435/264 , 435/325 , 435/4 , 435/410 , 435/419 , 800/295 , 800/8

ABSTRACT:

The present invention provides pharmaceutical preparations for anti-inflammation, anti-infection, inhibition of cancer metastasis etc., foods such as dairy products etc., and a method for improving proteins, as well as a method for diagnosis of inflammatory diseases and cancer malignancy. According to the present invention, there can be provided a polypeptide having poly-N-acetyllactosamine sugar chains synthesis-related activity, a process for producing the polypeptide, DNA coding for the polypeptide, a process for producing the DNA, a recombinant vector having the DNA integrated therein, a transformant carrying the recombinant vector, an antibody recognizing the polypeptide, a process for producing poly-N-acetyllactosamine sugar chains by use of the DNA or the polypeptide, diagnosis and treatment of diseases such as inflammations, cancers etc. by use of the DNA, the polypeptide or the antibody, determination and immunostaining of the polypeptide of the present invention by use of the antibody, a method for screening a compound varying the expression of a gene coding for the polypeptide, and a method for screening a substance varying the activity of the polypeptide.

30 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

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Brief Summary Text - BSTX (7):

The poly-N-acetyllactosamine sugar chain is synthesized by alternately reaction of .beta.1, 4-galactosyltransferases and .beta.1, 3-N-acetylglucosaminyltransferases. The gene coding for the former enzyme .beta.1,4-galactosyltransferase has already been cloned, but the gene coding for the latter enzyme .beta.1,3-N-acetylglucosaminyltransferase is still not cloned. With respect to .beta.1, 3-N-acetylglucosaminyltransferases having poly-N-acetyllactosamine synthesis-related activity, there are only reports on their partial purification resulting in no information of their amino acid sequences [J. Biol. Chem., 268, 27118 (1993), J. Biol. Chem., 267, 2994 (1992), J. Biol. Chem., 263, 12461 (1988), Jpn. J. Med. Sci. Biol., 42, 77 (1989)].

Brief Summary Text - BSTX (29):

If a core 2.beta.1, 6-N-acetylglucosaminyltransferase gene is expressed in T-cell line EL-4, the molecular weight of CD43, CD45 or CD44 as a membrane protein on cell surface is increased [J. Biol. Chem., 271, 18732 (1996)]. This would be because sugar chains synthesized by core 2.beta.1, 6-N-acetylglucosaminyltransferase serve as good substrates for .beta.1, 3-N-acetylglucosaminyltransferase involved in synthesizing poly-N-acetyllactosamine sugar chains.

Brief Summary Text - BSTX (33):

It is expected that inflammatory diseases or cancer malignancy can be diagnosed by examining expression of genes (e.g. a gene for .beta.1,3-N-acetylglucosaminyltransferase as a key enzyme in synthesis of poly-N-acetyllactosamine sugar chains) involved in synthesizing poly-N-acetyllactosamine sugar chains or by examining expression of polypeptides coded by their genes in inflammatory leukocytes, cancer cells or in serum. However, such a method is not known.

Other Reference Publication - OREF (3):

Holmes E et al. Characterization of a beta 1-3-N-acetylglucosaminyltransferase associated with synthesis of type 1 and type 2 lacto-series tumor-associated antigen from the human colonic adenocarcinoma cell line SW403, Archives of Biochem. and Biophys., J, Dec. 1993.

US-PAT-NO: 6131578

DOCUMENT-IDENTIFIER: US 6131578 A

TITLE: Inhibitors of UDP-G1cNAc:Ga1.beta.1-3Ga1NAc.alpha.R .beta.1-6 N-acetylglucosaminyltransferase (core 2 G1cNAc-T) and use of the inhibitors to prevent or treat cardiomyopathy associated with diabetes

DATE-ISSUED: October 17, 2000

INVENTOR-INFORMATION:

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APPL-NO: 08/ 943058

DATE FILED: October 2, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of provisional application 60/046,876 filed Oct. 2, 1996 now abandoned.

US-CL-CURRENT: 128/898, 514/44

ABSTRACT:

Cardiomyopathy associated with diabetes and hyperglycemia can be treated by administering to a subject suffering from this condition a substance that inhibits UDP-GlcNAc:Gal.beta.1-3GalNAc.alpha.R.beta.1-6-N-acetylglucosaminyl transferase (core 2 GlcNAc-T) activity.

10 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Brief Summary Text - BSTX (10):

Therefore, broadly stated the present invention relates to a method of preventing or treating cardiomyopathy associated with diabetes and hyperglycemia in a subject comprising reducing core 2 GlcNAc-T activity. Levels of core 2 GlcNAc-T activity may be reduced by administering a substance which inhibits core 2 GlcNAc-T activity. Substances which inhibit core 2 GlcNAc-T activity include known inhibitors of core 2 GlcNAc-T activity, inhibitors identified using the methods described herein, and antisense

sequences of a nucleic acid sequence encoding core 2 GlcNAc-T activity.

Brief Summary Text - BSTX (14):
Substances which inhibit transcription or translation of the gene encoding core 2 GlcNAc-T may be identified by transfecting a cell with an expression vector comprising a recombinant molecule containing a nucleic acid sequence encoding core 2 GlcNAc-T, the necessary elements for the transcription and/or translation of the nucleic acid sequence and a reporter gene, in the presence of a substance suspected of inhibiting transcription or translation of the gene encoding core 2 GlcNAc-T activity, and comparing the level of expression of core 2 GlcNAc-T or the expression of the protein encoded by the reporter gene with a control cell transfected with the nucleic acid molecule in the absence of the substance. The method can be used to identify transcription and translation inhibitors of the gene encoding core 2 GlcNAc-T.

Drawing Description Text - DRTX (5):
FIG. 3 shows the nucleotide and deduced amino acid sequence of DH1 identifying the sequence as core 2 GlcNAc-T;

Drawing Description Text - DRTX (13):
FIG. 9 shows the nucleotide and amino acid sequence of human core 2 GlcNAc-T.

Detailed Description Text - DETX (2):
As discussed above, the present invention relates to a method of preventing or treating cardiomyopathy associated with diabetes and hyperglycemia comprising reducing core 2 GlcNAc-T activity. Levels of core 2 GlcNAc-T activity may be reduced by administering a substance which inhibits core 2 GlcNAc-T activity, or inhibits transcription or translation of the gene

Detailed Description Text - DETX (5):
Recombinant molecules containing the nucleic acid sequence of core 2 GlcNAc-T in antisense orientation may be used to inhibit core 2 GlcNAc-T activity. The nucleic acid sequence shown in FIG. 9 (see also GenBank Accession Nos. L41415, U41320, and U19265), or parts thereof, may be inverted relative to their normal presentation for transcription to produce antisense nucleic acid molecules. The antisense nucleic acid molecules may be contructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. The antisense nucleic acid molecules or a part thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules, or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothiate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

Detailed Description Text - DETX (9):
The core 2 GlcNAc-T may be obtained from commercial sources, or prepared by expression of the gene encoding core 2 GlcNAc-T in host cells (for example, transfected CHO cells). A substance that inhibits core 2 GlcNAc-T activity may also be identified by treating a cell which expresses core 2 GlcNAc-T with a substance which is suspected of affecting core 2 GlcNAc-T activity, and assaying for Gal.beta.1-3[GalNAc.beta.1-6]GalNAc.alpha. associated with the cell. Gal.beta.1-3[GalNAc.beta.1-6]GalNA.alpha. can be measured using a substance that binds to the oligosaccharide product either alone or in association with an attached glycoprotein. For example, cells expressing core

2 GlcNAc-T may be treated with a substance suspected of inhibiting core 2 GlcNAc-T activity. An antibody specific for the oligosaccharide product can be added and the amount of antibody binding can be compared to control cells which have not been treated with the substance and/or which do not express core 2 GlcNAc-T. Antibodies specific for core 2 GlcNAc-T may be obtained from commercial sources, for example 1B11 rat anti-mouse CD43 activation-associated isoform monoclonal antibody supplied by Pharmingen Inc.

Detailed Description Text - DETX (10):
Substances which inhibit core 2 GlcNAc-T include substances which inhibit transcription or translation of the gene encoding core 2 GlcNAc-T. Transcription inhibitors may be identified by transfecting a host cell with a recombinant molecule comprising a nucleic acid sequence encoding core 2 GlcNAc-T, the necessary elements for the transcription of the nucleic acid sequence, and a reporter gene, in the presence of a substance suspected of inhibiting transcription of the gene encoding core 2 GlcNAc-T, and comparing the level of mRNA or expression of the protein encoded by the reporter gene with a control cell transfected with the nucleic acid molecule in the absence of the substance. Translation inhibitors may be identified by transfecting a host cell with a recombinant molecule comprising a nucleic acid sequence encoding core 2 GlcNAc-T, the necessary elements for the transcription and translation of the nucleic acid sequence, and a reporter gene, in the presence of a substance suspected of inhibiting translation of the gene encoding core 2 GlcNAc-T, and comparing the level of expression of core 2 GlcNAc-T with a control cell transfected with the nucleic acid molecule in the absence of the substance.

Detailed Description Text - DETX (11):
A recombinant molecule comprising a nucleic acid sequence encoding core 2 GlcNAc-T may be constructed having regard to the sequence of the core 2 GlcNAc-T gene (see FIG. 9) using chemical synthesis and enzymatic ligation reactions following procedures known in the art.

Detailed Description Text - DETX (13):
Examples of reporter genes are genes encoding a protein such as .beta.-galactosidase (e.g. lac Z), chloramphenicol, acetyl-transferase, firefly luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. Transcription of the reporter gene is monitored by changes in the concentration of the reporter protein such as .beta.-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. This makes it possible to visualize and assay for expression of recombinant molecules to determine the effect of a substance on expression of the core 2 GlcNAc-T gene.

Detailed Description Text - DETX (24):
In accordance with one embodiment of the invention, the transgenic non-human animal contains a DNA construct comprising a gene encoding core 2 GlcNAc-T. In accordance with a preferred embodiment of the invention, the transgenic non-human animal contains a DNA construct comprising a gene encoding core 2 GlcNAc-T and a promoter which stimulates expression of the gene in the cardiovascular system. Suitable promoters include the cardiac myosin promoter.

Detailed Description Text - DETX (46):
Cloning full-length DH1 cDNA. To facilitate identification, a cDNA library derived from diabetic rat heart mRNA was screened using the 214-bp-cloned DH1 PCR fragment as a probe. Five overlapping recombinants were identified and the composite of the full cDNA sequence was determined (FIG. 3(A)). It contained 5,010 bp inclusive of poly A tail and corresponded to the size detected by the original Northern blot analysis. Open reading frame analysis showed that the

longest possible coding region which was from position 802 to 2085 and encoded 428 amino acids. The GXXATGC pattern was observed flanking the region of the presumptive start codon (31) and a polyadenylation signal, AATAAA, was found 15 bp upstream from the polyA tail. Searches for homologous sequences in Genbank/EMBL revealed that this cDNA shared 80% identity at the nucleotide level and 85% identity at the amino acid level with human core 2 (GlcNAc-T) (32). The mouse core 2 GlcNAc-T was also cloned and sequenced and it was found that DH1 shared 92% identity with the amino acid sequence of mouse core 2 GlcNAc-T (FIG. 3(B)). These findings strongly suggested that DH1 was rat core 2 GlcNAc-T.

Detailed Description Text - DETX (88):

32. Bierhuizen. M. F., and M. Fukuda. 1992. Expression cloning of a cDNA encoding UDP-GlcNAc:GalB1-3GalNAc-R(GlcNAc to GalNAc) B1-GlcNAc transferase by gene transfer into CHO cells expressing polyoma large tumor antigen. Proc. Natl. Acad Sci. USA. 89:9326-9330.

Claims Text - CLTX (2):

2. A method as claimed in claim 1, wherein the substance is an antisense sequence of a nucleic acid sequence encoding a protein with core 2 GlcNAc-T activity.

Claims Text - CLTX (3):

3. A method as claimed in claim 2, wherein the substance is an antisense nucleic acid molecule of a core 2 GlcNAc-T sequence as shown in FIG. 9 or GenBank Accession Nos. L41415, U41320, or U19265.

Claims Text - CLTX (9):

9. A method as claimed in claim 1, wherein the substance which inhibits core 2 GlcNAc-T activity is an inhibitor of transcription or translation of a gene encoding a protein with core 2 GlcNAc-T activity.

Claims Text - CLTX (10):

10. A method as claimed in claim 1, wherein the substance which inhibits core 2 GlcNAc-T activity is an inhibitor of translation of a gene encoding a protein with core 2 GlcNAc-T activity.

US-PAT-NO: 6096725

DOCUMENT-IDENTIFIER: US 6096725 A

TITLE: Methods of using .alpha.Gal oligosaccharides as immune system targeting agents

DATE-ISSUED: August 1, 2000

INVENTOR-INFORMATION:

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APPL-NO: 08/ 887270

DATE FILED: July 2, 1997

US-CL-CURRENT: 514/53, 424/137.1 , 514/54 , 514/62

ABSTRACT:

The invention relates to methods for attenuating xenograft rejection in humans and old world monkeys, using oligosaccharides containing a Gal.alpha.1-3Gal motif, to neutralize or remove anti-.alpha.Gal antibodies. The invention additionally relates to methods for site directed activation of the complement cascade or host leukocytes using oligosaccharides containing a Gal.alpha.1-3Gal motif to target anti-.alpha.Gal antibodies. The invention further relates to pharmaceutical compositions that may be used in the practice of the invention. Such compositions contain, as the active ingredient, an oligosaccharide containing a Gal.alpha.1-3Gal motif effective in binding anti-.alpha.Gal antibodies in vivo or ex vivo.

8 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Detailed Description Text - DETX (37):
In another example, the .alpha.Gal pentasaccharide (Gal.alpha.1-3Gal.beta.1-4GlcNAc.beta.1-3Gal.beta.1-4Glc) is synthesized by contacting lactose (galactose.beta.1-4glucose) with UDP-N-acetylglucosamine and a galactoside .beta.1-3 N-acetylglucosaminyl transferase. The product trisaccharide is contacted with UDP-Gal and a .beta.-N-acetylglucosaminoside .beta.1-4 galactosyltransferase and the resulting tetrasaccharide is contacted with UDP-galactose and a .beta.-galactoside .beta.1-3 galactosyltransferase. The resulting pentasaccharide is concentrated using techniques known in the art (See, for example, Section 6.2).

Detailed Description Text - DETX (134):
Lactose was dissolved in 5 mM sodium phosphate buffer at mM, and UDP-GlcNAc, obtained by fermentation, using techniques known in the art, was then added to a final concentration of 5 mM. A bacterial lysate containing recombinant

Neisseria polysaccharea .beta.1,3-N-acetylglucosaminyltransferase was then added to this mixture to a final activity of 10 .mu.moles/min/L. The DNA encoding this .beta.1,3-N-acetylglucosaminyltransferase was generated by the polymerase chain reaction (PCR) using techniques known in the art, with primers corresponding to sequences flanking the .beta.1,3--N-acetylglucosaminyltransferase of Neisseria gonorrhoea (see U.S. Pat. No. 5,545,553) and Neisseria polysaccharea template DNA that was isolated using standard methods known in the art. The PCR generated DNA was then cloned into the pGEX expression vector (Pharmacia) using methods known in the art. The protein expressed by this expression construct is a fusion protein in which glutathione-S-transferase is fused to the amino terminal end of the .beta.1,3-N-acetylglucosaminyltransferase. Lysates containing this fusion protein were prepared from E. coil host cells that were transformed with the expression construct and in which expression of the construct had been induced through the addition of IPTG.

US-PAT-NO: 6015701

DOCUMENT-IDENTIFIER: US 6015701 A
See image for Certificate of Correction

TITLE: N-acetylglucosaminyltransferase V proteins and coding sequences

DATE-ISSUED: January 18, 2000

INVENTOR-INFORMATION:

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APPL-NO: 08/ 276968

DATE FILED: July 19, 1994

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 08/016,863, filed Feb. 10, 1993, now U.S. Pat. No. 5,602,003, which application is a continuation-in-part of U.S. Ser. No. 07/905,795, filed Jun. 29, 1992, now abandoned.

US-CL-CURRENT: 435/193, 435/252.3 , 435/252.33 , 435/320.1 , 435/325 , 536/23.2

ABSTRACT:

The present invention provides substantially purified UDP-N-acetylglucosamine: .alpha.-6-D-mannoside .beta.-1,6-N-acetylglucosaminyl transferase (GlcNAc T-V; EC 2.4.1.155) proteins and antibodies which specifically bind GlcNAc T-V. The present invention also provides polynucleotide sequences and oligonucleotide probes capable of specifically hybridizing to nucleic acid sequences which encode GlcNAc T-V, and cDNA and genomic clones encoding GlcNAc T-V, as well as nucleotide sequences encoding GlcNAc T-V, as specifically exemplified by GlcNAc T-V coding sequences from rat, hamster, mouse and human.

31 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 20

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Abstract Text - ABTX (1):
The present invention provides substantially purified UDP-N-acetylglucosamine: .alpha.-6-D-mannoside .beta.-1,6-N-acetylglucosaminyl transferase (GlcNAc T-V; EC 2.4.1.155) proteins and antibodies which specifically bind GlcNAc T-V. The present invention also provides

polynucleotide sequences and oligonucleotide probes capable of specifically hybridizing to nucleic acid sequences which encode GlcNAc T-V, and cDNA and genomic clones encoding GlcNAc T-V, as well as nucleotide sequences encoding GlcNAc T-V, as specifically exemplified by GlcNAc T-V coding sequences from rat, hamster, mouse and human.

TITLE - TI (1):
N-acetylglucosaminyltransferase V proteins and coding sequences

Brief Summary Text - BSTX (2):
The field of this invention is the area of protein glycosylation, specifically the area of the particular enzyme, UDP N-acetylglucosaminyltransferase V, involved in the expression of the .beta.(1,6) branch structure found in tri- and tetraantennary N-linked oligosaccharides. The field relates to purified active enzyme, the amino acid sequences of rat, human and hamster GlcNAc T-V proteins, genes encoding active enzyme and cell lines genetically engineered to express a nucleotide sequence encoding active enzyme.

Brief Summary Text - BSTX (13):
Additional aspects of the present invention are genetically engineered, soluble GlcNAc T-V enzymatically active proteins, as exemplified herein by a soluble GlcNAc T-V derived from the rat sequence, which has Gln at the N-terminus, followed by an amino acid sequence as given in SEQ ID NO:16, amino acid 70 through amino acid 741. Also within the present invention are nucleic acid molecules genetically engineered to produce soluble GlcNAc T-V proteins from cell-free culture media. Preferably, EDTA is present during purification steps to prevent proteolytic degradation; preferred purification steps are copper chelating column chromatography and CM Sephadex chromatography.

Brief Summary Text - BSTX (14):
Also embodied in the invention are genomic and cDNA sequences encoding glcNAc T-V, the amino acid sequences of GlcNAc T-V enzymes, and recombinant host cells genetically engineered to express sequences encoding active GlcNAc T-V enzymes.

Brief Summary Text - BSTX (15):
Also provided by this invention are polyclonal and monoclonal antibodies specific for GlcNAc T-V. These antibodies will also bind to and be useful for detection and isolation of GlcNAc T-V from mammalian and other sources. It is understood that the molecular weight, kinetic parameters and primary amino acid sequence of GlcNAc T-V from a source other than rat kidney may vary from those values disclosed herein for the rat kidney enzyme.

Brief Summary Text - BSTX (16):
Also provided in this invention is GlcNAc T-V produced by recombinant DNA technology in prokaryotic or eukaryotic host cells. Disclosed in this invention are the complete amino acid sequences for rat, human and hamster (e.g., Chinese Hamster Ovary (CHO) cells) GlcNAc T-V and nucleotide sequences encoding rat, human and hamster GlcNAc T-V. Examples of methods of producing recombinant active GlcNAc T-V by recombinant DNA technology are disclosed. The exemplified amino acid sequences and the nucleotide sequences encoding GlcNAc T-V, and subsequences within, as understood in the art, will be useful for isolating GlcNAc T-V coding sequences from a wide range of species and for producing useful quantities of GlcNAc T-V by recombinant DNA technology.

Brief Summary Text - BSTX (17):
Further objects of this invention are cDNA clones encoding GlcNAc T-V and genomic clones encoding GlcNAc T-V. The antibodies raised against rat kidney

GlcNAc T-V (or other GlcNAc T-V's or peptide-specific antibodies for GlcNAc T-V) can be used to detect expression of GlcNAc T-V from sources other than rat kidney by virtue of cross-reactivity with those other GlcNAc T-V enzymes; alternatively, these antibodies can be used to screen cDNA expression libraries. Sequences encoding GlcNAc T-V from rat, human and hamster (i.e., Chinese hamster ovary) cells and a partial coding sequence from mouse are presented herein. Similarly, the degenerate oligonucleotide probes and/or the coding sequence and/or the amplimer sequences of the present invention can be used to screen genomic or cDNA libraries constructed using nucleic acids from sources other than those exemplified herein, or these can be used to prepare primers to amplify sequences encoding GlcNAc T-V from mRNA populations prepared from rat kidney or from other animal cells. The cDNA and/or genomic sequences encoding GlcNAc T-V will be useful in directing the recombinant expression of GlcNAc T-V.

Brief Summary Text - BSTX (18):

Further objects of this invention are nucleotide sequences encoding rat GlcNAc T-V, and nucleotide sequences encoding GlcNAc T-V from other vertebrate, preferably mammalian, sources, including cDNA and genomic sequences. The nucleotide sequence encoding rat GlcNac T-V is provided herein as SEQ ID NO:15, from an ATG translation start codon beginning at nucleotide 299 through a translation stop codon ending at nucleotide 2521. The nucleotide sequence encoding human GlcNac T-V is provided herein as SEQ ID NO:19, from an ATG translation start codon beginning at nucleotide 38 through a translation stop codon ending at nucleotide 2263. The nucleotide sequence encoding hamster (i.e., CHO cells) GlcNac T-V is provided herein as SEQ ID NO:17, from an ATG translation start codon beginning at nucleotide 145 through a translation stop codon ending at nucleotide 2367. A partial mouse cDNA sequence is given in SEQ ID NO:21.

Brief Summary Text - BSTX (19):

The skilled artisan recognizes that there will be more than one nucleotide sequence capable of encoding the same amino acid sequence due to the degeneracy of the genetic code. Exemplary GlcNAc T-V amino acid sequences are given in SEQ ID NOs 16, 18 and 20. These sequences, and sequence variants thereof which encode functionally equivalent GlcNAc T-V, can be used to express GlcNAc T-V in a desired recombinant host cell. The GlcNAc T-V coding sequences from other vertebrate species, preferably from mammals, will be highly homologous at the nucleotide sequence level to the exemplified rat, hamster and human GlcNAc T-V coding sequence disclosed herein. Functionally equivalent GlcNAc T-V coding sequences with at least 70%, preferably at least 80%, more preferably at least 90% nucleotide sequence homology to the exemplified rat, human and/or hamster (CHO) GlcNAc T-V coding sequences can be identified and isolated from cDNA libraries prepared from mRNA sources other than rat, human and CHO cells, using well-known DNA-DNA hybridization technology and the exemplified GlcNAc T-V coding sequences provided herein. Also contemplated are genomic clones encoding GlcNAc T-V, which clones comprise the natural regulatory sequences. It is understood that any intron sequences in genomic GlcNAc T-V are not to be included in sequence comparisons to the exemplified full-length coding sequence, and gaps may be introduced to maximize homology.

Brief Summary Text - BSTX (20):

Additional objects of this invention are DNA molecules containing a first nucleotide sequence encoding an enzymatically active GlcNAc T-V and a second nucleotide sequence not found associated with the GlcNAc T-V coding sequence in nature, termed an exogenous nucleotide sequence herein. Preferably the first nucleotide sequence encodes a polypeptide sequence with GlcNAc T-V activity, said polypeptide having an amino acid sequence as given in FIG. 12 and in SEQ ID NOS:16, 20 and 18 (from rat, human and CHO cells, respectively).

Brief Summary Text - BSTX (21):
Still further objects of the invention are cells genetically engineered to contain a DNA molecule containing a first nucleotide sequence encoding an enzymatically active GlcNAc T-V and a second nucleotide sequence not found associated with the GlcNAc T-V coding sequence in nature. Mammalian cells are preferred for recombinant expression of GlcNAc T-V coding sequences. Particularly preferred are COS-7 cells and CHO (Chinese Hamster Ovary) cells. The exemplified rat, CHO and human GlcNAc T-V amino acid sequences are particularly preferred, preferably encoded by the exemplified nucleotide coding sequences as in FIG. 11 or SEQ ID NO:15 from nucleotide 299 through nucleotide 2521, in SEQ ID NO:17 from nucleotide 145 through nucleotide 2367, and in SEQ ID NO:19 from nucleotide 38 through nucleotide 2263.

Drawing Description Text - DRTX (5):
FIG. 4 is a reproduction of an ethidium bromide-stained agarose gel, showing the reaction products of the results of PCR amplification of GlcNAc T-V coding sequences. Lane 1 contains molecular weight standards (123 ladder); Lanes 2 and 7 were the results of reactions containing mouse lymphoma cell line BW5147 cDNA from total RNA as a template; Lanes 3 and 8 were the results of reactions containing mouse lymphoma cell line BW5147 cDNA from poly(A)+ RNA as template; Lanes 4 and 9 were the results of reactions containing rat mammary tumor cell line MAT C1 cDNA from total RNA as template; Lanes 5 and 10 were the results of reactions containing rat mammary tumor cell line MAT C1 cDNA from poly(A)+ RNA as template; and Lanes 6 and 11 were the results for reactions without added template. The reactions run in Lanes 2-6 were carried out with Primer 1 (SEQ ID NO:5) and Antiprimer 2 (SEQ ID NO:8) as the primers for PCR. In the reactions run in Lanes 7-11 were carried out with Primer 2 (SEQ ID NO:7) and Antiprimer 1 (SEQ ID NO:6).

Drawing Description Text - DRTX (11):
FIGS. 10A-10E, taken together in sequence, presents the cDNA sequence encoding rat GlcNAc T-V and the deduced amino acid sequence which correspond to SEQ ID NO:15 and SEQ ID NO:16, respectively.

Drawing Description Text - DRTX (13):
FIG. 12A-12B, taken in sequence, presents a comparison of the deduced amino acid sequences of human (SEQ ID NO:20), CHO (SEQ ID NO:18), mouse (SEQ ID NO:22) and rat (SEQ ID NO:16) GlcNAc T-V. The human sequence contains an insertion of a valine at amino acid 109 as compared to the CHO and rat sequences. The mouse sequence represents the analysis of a partial cDNA clone starting at amino acid 288 according to the numbering in SEQ ID NO:16. The asterisks signify the end of the protein.

Detailed Description Text - DETX (4):
Complementary DNA (cDNA) synthesis involves the in vitro synthesis of a double stranded DNA sequence by enzymatic reverse transcription of mRNA isolated from donor cells. In the present invention, polyadenylated RNA is prepared from rat 1-EJ cultured cells (described in Peles et al. (1992) Cell 69:205-216). Rat 1-EJ cells are Rat 1 fibroblasts which have been transfected with the human EJ gene, an activated Harvey ras gene, which is believed to elevate expression levels for GlcNAc T-V. cDNA molecules and/or libraries can be used for isolating a DNA sequence encoding a selected protein when the entire amino acid sequence of that protein is not known. Isolating a gene from a cDNA library is made much easier when at least a partial amino acid sequence is known, and is further facilitated when a complete coding sequence from at least one species is known. Procedures for the preparation of cDNA sequences in plasmid libraries derived from the reverse transcription of mRNA are well-known to the art.

Detailed Description Text - DETX (7):
The term expression control sequences refer to DNA sequences that control and regulate the transcription and translation of another DNA sequence (i.e., a coding sequence). A coding sequence is operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that coding sequence. Expression control sequences include, but are not limited to, promoters, enhancers, promoter-associated regulatory sequences, transcription termination and polyadenylation sequences, and their positioning and use is well understood by the ordinary skilled artisan. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene. The combination of the expression control sequences and the GlcNAc T-V coding sequences form the GlcNAc T-V expression cassette.

Detailed Description Text - DETX (8):
As used herein, an exogenous nucleotide sequence is one which is not in nature covalently linked to a particular nucleotide sequence, e.g., a GlcNAc T-V coding sequence. Examples of exogenous nucleotide sequences include, but are not limited to, plasmid vector sequences, expression control sequences not naturally associated with particular GlcNAc T-V coding sequences, and viral vector sequences. A non-naturally occurring DNA molecule is one which does not occur in nature, and it is thus distinguished from a chromosome, for example. As used herein, a non-naturally occurring DNA molecule comprising a sequence encoding an expression product with GlcNAc T-V activity is one which comprises said coding sequence and sequences which are not associated therewith in nature.

Detailed Description Text - DETX (9):
Similarly, as used herein an exogenous gene is one which does not naturally occur in a particular recombinant host cell but has been introduced in using genetic engineering techniques well known in the art. An exogenous gene as used herein can comprise a GlcNAc T-V coding sequence expressed under the control of an expression control sequence not associated in nature with said coding sequence.

Detailed Description Text - DETX (10):
Another feature of this invention is the expression of the sequences encoding GlcNAc T-V. As is well-known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate host cell.

Detailed Description Text - DETX (18):
It is understood by those skilled in the art that the exemplified rat GlcNAc T-V coding sequence, provided herein in FIG. 10 and in SEQ ID NO:15 from nucleotide 299 through nucleotide 2521, is representative of GlcNAc T-V from other vertebrate sources, especially of other mammalian sources, including humans. SEQ ID NO:17 and SEQ ID NO:19 provide the CHO and human sequences, respectively, encoding GlcNAc T-V, and SEQ ID NO:21 provides a partial mouse sequence encoding GlcNAc T-V. The coding sequences for GlcNAc T-V provided herein are suitable for use in preparing or deriving PCR primers for identifying and/or amplifying sequences encoding human or other animal GlcNAc T-V, and/or for use as hybridization probes to identify clones encoding human,

hamster, rat, other mammalian or other vertebrate GlcNAc T-V in appropriate genomic or cDNA libraries.

Detailed Description Text - DETX (20):
Species other than rat, mouse, hamster and human contain genes encoding proteins which catalyze the same enzymatic reaction as rat GlcNAc T-V, which genes have significant sequence homology to the rat, hamster, mouse and human sequences encoding GlcNAc T-V. One can isolate these homologous cDNAs and/or genes using the DNA sequences of this invention as probes or primers under standard hybridization conditions. This invention specifically contemplates and encompasses such sequences.

Detailed Description Text - DETX (21):
A comparison of the human, CHO, rat and partial mouse GlcNAc T-V nucleotide sequences is presented in FIGS. 11A-11F (SEQ ID NOS:19, 17, 15 and 21, respectively). The coding region of SEQ ID NO:15 extends from an ATG starting at nucleotide 299 to a stop codon ending at nucleotide 2524. The rat sequence contains 298 bp of upstream 5' untranslated sequence. The human and the CHO sequences contain 136 bp and 243 bp of 5' untranslated sequence respectively. The partial mouse sequence is presented starting within the coding region at the nucleotide numbered 1159 of SEQ ID NO:15. In addition, approximately 300 bp of the human, 100 bp of the rat and 325 bp of the mouse 3' untranslated regions are provided. Analysis of the coding regions of these sequences indicates that there is approximately 89% homology of the human sequence compared with the rat sequence. The CHO sequence shares an approximately 93% homology with the rat sequence. In a comparison of the partial mouse coding region with the corresponding portion of the rat sequence, approximately 96% nucleotide sequence homology is obtained.

Detailed Description Text - DETX (22):
In FIGS. 12A-12B the human (SEQ ID NO:20), CHO (SEQ ID NO:18), rat (SEQ ID NO:16) and partial mouse (SEQ ID NO:22) GlcNAc T-V deduced amino acid sequences are compared. The partial mouse sequence is presented starting at amino acid 288 of SEQ ID NO:16. The human GlcNAc T-V sequence contains an additional valine at amino acid 109 compared to the rat and CHO sequences. The available mouse sequence does not extend to this region. The additional amino acid in the human sequence occurs at the site of the first potential N-linked glycosylation site, although the potential glycosylation sequence is maintained. The human, CHO and rat sequences all contain the same six potential N-glycosylation sites. The mouse sequence also shares the three potential N-glycosylation sites that are located within the available GlcNAc T-V sequence. There is approximately 98% amino acid sequence identity between human and rat amino acid sequences. The CHO amino acid sequence is approximately 99% identical with the rat, and the mouse amino acid sequence is greater than 99% identical with the rat over the region for which the mouse sequence was obtained.

Detailed Description Text - DETX (23):
Thus, GlcNAc T-V coding sequences from vertebrate sources have significant sequence homology to the exemplified rat, human and hamster GlcNAc T-V coding sequences and the encoded GlcNAc T-V enzymes have a high degree of amino acid sequence identity as disclosed herein. It is obvious to one normally skilled in the art that human, rat and CHO GlcNAc T-V cDNA clones, genomic clones and PCR ampliners can be readily isolated using standard procedures and the sequence information provided herein. There would be no need to practice these examples exactly, but rather the sequence information provided herein (SEQ ID NOs 15-22) enables the isolation of rat, CHO, mouse, human and other GlcNAc T-V nucleic acid coding sequences and amino acid sequences. It is further obvious to one normally skilled in the art that, as demonstrated in Examples 12 and 13,

GlcNAc T-V cDNA and genomic clones, cDNA and genomic gene sequences, and amino acid sequences can be readily obtained and used for GlcNAc T-V from any mammalian species using standard procedures and the sequence information provided herein. The ordinary skilled artisan can utilize the exemplified sequences provided herein, or portions thereof, preferably at least 25-30 bases in length, in hybridization probes to identify cDNA (or genomic) clones encoding GlcNAc T-V, where there is at least 70% sequence homology to the probe sequence using appropriate art-known hybridization techniques. The skilled artisan understands that the capacity of a cloned cDNA to encode functional GlcNAc T-V enzyme can be readily tested as taught herein (See Example 11).

Detailed Description Text - DETX (24):
Hybridization conditions appropriate for detecting various extents of nucleotide sequence homology between probe and target sequences and theoretical and practical consideration are given, for example in B. D. Hames and S. J. Higgins (1985) Nucleic Acid Hybridization, IRL Press, Oxford, and in Sambrook et al. (1989) supra. Under particular hybridization conditions the DNA sequences of this invention will hybridize to other DNA sequences having sufficient homology, including homologous sequences from different species. It is understood in the art that the stringency of hybridization conditions is a factor in the degree of homology required for hybridization. The skilled artisan knows how to manipulate the hybridization conditions so that the stringency of hybridization is at the desired level (high, medium, low). If attempts to identify and isolate the GlcNAc T-V gene from another mammalian source fail using high stringency conditions, the skilled artisan will understand how to decrease the stringency of the hybridization conditions so that a sequence with a lower degree of sequence homology will hybridize to the sequence used as a probe. The choice of the length and sequence of the probe is readily understood by the skilled artisan.

Detailed Description Text - DETX (25):
When a cDNA library is used as a source of GlcNAc T-V coding sequences, the skilled artisan will take steps to insure that the library is of high quality, i.e., that rare mRNAs will be represented and that large mRNAs (larger than about 3 kb) will be present as full length cDNA clones. If the artisan uses one of the commercially available or otherwise accessible cDNA libraries, he will choose one that meets the criteria taught herein. Providing for rare and/or large message representation is within the skill of the art.

Detailed Description Text - DETX (26):
The DNA sequences of this invention refer to DNA sequences prepared or isolated using recombinant DNA techniques. These include cDNA sequences, sequences isolated using PCR, DNA sequences isolated from their native genome, and synthetic DNA sequences. As used herein, this term is not intended to encompass naturally-occurring chromosomes or genomes. Sequences derived from the GlcNAc T-V gene can be used in studying the regulation of GlcNAc T-V expression in normal cells, in transformed cells and in metastatic tumor cells, and can be used in designing mechanisms, e.g., via antisense RNA or DNA, for inhibiting metastasis of tumor cells. These sequences can also be used to direct recombinant synthesis of GlcNAc T-V.

Detailed Description Text - DETX (40):
These amino acid sequences were confirmed by comparison with the deduced amino acid sequence of the rat GlcNAc T-V, and it was deduced that the Glu residue of SEQ ID NO:4 should be Gly. The amino acid sequences obtained from the four peaks were searched within the Swiss Protein Data Bank and deduced degenerate coding sequences were searched in the Genbank database. No significantly homologous sequences were found.

Detailed Description Text - DETX (41):
The determination of a partial amino acid sequence for GlcNAc T-V allows the production of sets of degenerate oligonucleotide probes or primers, thus, enabling the cloning of the corresponding cDNA and genomic clones. Those oligonucleotides can also be used to study the transcriptional and/or translational mechanisms which control the level of expression of the gene encoding GlcNAc T-V.

Detailed Description Text - DETX (42):
From the amino acid sequences for the internal peptides corresponding to peaks 34 and 49, corresponding degenerate oligonucleotides were designed for use as primers for PCR amplification of cDNA sequences encoding GlcNAc T-V. The degenerate 29 base oligonucleotide designed from the sequence of first ten amino acids of the Peak 34 peptide is presented as Primer 1 (SEQ ID NO:5). The antisense counterpart (SEQ ID NO:6) of Primer 1, termed antiprimer 1 herein, will be useful as a primer in the PCR amplification of sequences encoding GlcNAc T-V present within polyadenylated mRNA populations, prepared from cells including, but not limited to, rat kidney, mouse lymphoma BW5147 cells and ascites-grown rat mammary gland tumor MAT C1 cells.

Detailed Description Text - DETX (48):
The antisense primers given above can also be used to amplify mRNA encoding GlcNAc T-V in polymerase chain reactions. Other oligonucleotide primers and "antiprimers" may be designed using the peptide sequences and/or GlcNAc T-V sequences disclosed herein by one of ordinary skill in the art for use in priming PCR synthesis of GlcNAc T-V coding sequences.

Detailed Description Text - DETX (50):
For PCR amplification of sequences encoding GlcNAc T-V, Primer 1 and AntiPrimer 2 cDNA were used to prime PCR-directed DNA synthesis. The combination of Primer 2 (SEQ ID NO:7) and AntiPrimer 1 (SEQ ID NO:6) did not yield an amplification product from either cell line. Using cDNA prepared from poly(A)+ RNA from either the rat mammary tumor line MAT C1 or from the mouse lymphoma cell line BW5147 with Primer 1 and AntiPrimer 2, an amplification product of about 200 bp was obtained, as shown in FIG. 4. These results indicate that the peak 34 sequence (SEQ ID NO:1) is located about 60 amino acids toward the amino end of the protein as compared with the peak 49 amino acid sequence. Background signal was reduced substantially by using 55.degree. C. rather than 50.degree. C. as the annealing temperature in the PCR reactions. The results also indicate a high degree of homology between the GlcNAc T-V coding sequences in mouse and rat. Thus, the primer/antiprimer sequences disclosed herein will be useful in identifying GlcNAc T-V genes and coding sequences of mammals other than rat.

Detailed Description Text - DETX (51):
The amplimer made by PCR with cDNA from MAT C1 poly(A)+ RNA as template and Primer 1 (SEQ ID NO:5) and AntiPrimer 2 (SEQ ID NO:8) was .sup.32 P-labelled for use as a hybridization probe. Rat MAT C1 genomic DNA and rat liver genomic DNA were digested in separate restriction endonuclease reactions, the fragments were separated in parallel using agarose gel electrophoresis, blotted to support and DNA-DNA hybridization was carried out under standard hybridization conditions of low stringency. Hybridization patterns were consistent with a single genetic locus encoding GlcNAc T-V in each. FIG. 5 illustrates the autoradiogram obtained for Southern hybridization with rat mammary tumor cell line MAT C1 and for rat liver genomic DNA. With BglII, BamHI/BglII and NcoI digestion, the size of the unique hybridizing genomic band is between 2 and 10 kbp. With NcoI/XhaI digestion, the size of the hybridizing band is between roughly 6 and 9 kb. Routine experimentation will allow size estimation with more precision. The 200 bp amplimer used in this experiment can be used to

screen cDNA or genomic libraries to identify GlcNAc T-V sequences. Standard "walking" experiments can be performed to obtain the sequences which flank the hybridizing fragment(s) after cloning of that fragment so that the entire gene can be isolated.

Detailed Description Text - DETX (52):
Labelled oligonucleotides having sequences of Primers 1 and 2 (SEQ ID NO: 5 and 7) or AntiPrimers 1 and 2 (SEQ ID NO:6 and SEQ ID NO:8), or preferably the PCR amplification product (amplimer) made using Primer 1 and AntiPrimer 2 as primers, can be successfully used as hybridization probes for screening cDNA libraries prepared from sources including mouse lymphoma BW5147 cells, mouse 3T3 cells and ascites-grown rat mammary gland MAT-C1 cells for sequences encoding GlcNAc T-V.

Detailed Description Text - DETX (53):
When a restriction fragment from within the coding region of a partial mouse cDNA clone was used as a hybridization probe in a Northern blot of rat kidney mRNA, a band of about 7 kb, along with apparent degradation products, was displayed (See FIG. 6). Thus, the size of the GlcNAc T-V MRNA is large, and care must be taken in preparing (or in choosing) a cDNA library from which to isolate a full length GlcNAc T-V coding sequence.

Detailed Description Text - DETX (54):
Examples 7-9 describe the steps in the successful identification and cloning of the rat GlcNAc T-V coding sequence using a PCR-cDNA strategy. In other experiments, an amplimer of about 170-200 bases was prepared by PCR. This amplimer was used to screen a mouse cDNA library, and a partial clone of about 1.7 kb was isolated. Sequence analysis revealed that the long open reading frame did not contain a start codon, and about 300 amino acids were determined by the open reading frame. A series of PCR amplification and screening steps were carried out using plasmid DNA prepared from pools of cDNA clones from subsets of a cDNA library prepared from Rat 1-EJ cell mRNA.

Detailed Description Text - DETX (55):
A rat cDNA clone of about 4.8 kb, carrying the full length GlcNAc T-V coding sequence was isolated. A portion of the cDNA was sequenced; that DNA sequence is presented in FIG. 10A-10E and in SEQ ID NO:15. The coding sequence extends from an ATG start codon beginning at nucleotide 299 through a stop codon ending at nucleotide 2521.

Detailed Description Text - DETX (56):
The deduced rat GlcNAc T-V amino acid sequence is given in FIG. 12A-12B and in SEQ ID NO:16. The predicted molecular weight of the encoded GlcNAc T-V, 84,561, is larger than the protein bands observed in and isolated from SDS-PAGE gels. A recent experiment has demonstrated that when GlcNAc T-V is purified from rat kidney by in the presence of a cocktail of protease inhibitors in vast excess, a band of about 95 kDa, in addition to the 69 and 75 kDa bands, is observed. When a radioactive photoaffinity active site label was used to tag active enzyme, all three bands were labelled. These observations suggest that the 75 and 69 kDa bands represent proteolytic fragments of the larger protein. The 95 kDa band is likely to represent a glycosylated form of the polypeptide encoded in SEQ ID NO:15. Six potential sites for N-linked glycosylation were identified: Asn residues at amino acid positions 109, 114, 117, 333, 432 and 446 in SEQ ID NO:16. A putative transmembrane domain, extending from amino acids 14-30, was identified by hydrophobicity analysis using Kyte and Doolittle methodology. This proposed transmembrane domain is characteristic of type II membrane proteins, and is similar to other enzymes of the lumen of the Golgi apparatus.

Detailed Description Text - DETX (57):
Within the deduced amino acid sequence of rat GlcNAc T-V (SEQ ID NO:16), the sequences corresponding to the Peak #s 34, 49 and 28 peptide sequences (SEQ ID NOs:1-3) were at amino acids 546-557, 592-607 and 375-386, respectively. The amino acid sequence of Peak #61 (SEQ ID NO:4) occurs at amino acids 168-177 in SEQ ID NO:16. The identities of the cysteine and aspartate residues are confirmed, and the amino acid at the ninth position in SEQ ID NO:4 was deduced to be glycine rather than glutamate, based on the nucleotide sequence in SEQ ID NO:15.

Detailed Description Text - DETX (59):
The 4.8 kb rat cDNA insert determined by partial DNA sequence analysis to contain an apparently full length GlcNAc T-V coding sequence was subcloned into the pJT-2 expression vector and electroporated into COS-7 cells (See Example 11). After 3 or 4 days incubation after the electroporation, the transfected cells were harvested, frozen and subsequently assayed for GlcNAc T-V activity. Parallel preparations of cells transfected with pJT-2 without insert DNA served as controls. It was estimated that about 3% of the cells were effectively electroporated. From the data in Table 2, it is clear that the cloned rat cDNA fragment encodes a functional GlcNAc T-V enzyme.

Detailed Description Text - DETX (60):
It will be a matter of routine experimentation for the ordinary skilled artisan to use the DNA sequence information presented herein to optimize GlcNAc T-V expression in a particular expression vector and cell line for a desired purpose. A cell line genetically engineered to contain and express a GlcNAc T-V coding sequence will be useful for the recombinant expression of protein products with the characteristic glycosylation dependent on GlcNAc T-V modification of glycoproteins. Any means known to the art can be used to introduce an expressible GlcNAc T-V coding sequence into a cell to produce a recombinant host cell, i.e., to genetically engineer such a recombinant host cell. Recombinant host cell lines which express high levels of GlcNAc T-V will be useful as sources for the purification of GlcNAc T-V, e.g., for studies of inhibitors of GlcNAc T-V activity for preventing or slowing metastasis of tumors. The coding sequence of GlcNAc T-V will be useful in preparing an antisense construct specific for GlcNAc T-V for inhibiting GlcNAc T-V expression where that is desired, for example, in metastasizing tumor cells.

Detailed Description Text - DETX (61):
Soluble secreted GlcNAc T-V enzyme proteins can be produced using the disclosure provided herein. A soluble GlcNA T-V is one which lacks the sequences in the amino terminal region of the protein which localize it to and bind it within the cell membrane, particularly within the Golgi apparatus. When the coding region of the enzymatically active portion of GlcNAc T-V, but not including the transmembrane region, is fused downstream of and in frame with a signal sequence coding sequence, and operably linked to transcriptional control sequences, and expressed in a suitable host cell, such as a mammalian cell, soluble GlcNAc T-V is expressed and secreted into the culture medium after the signal peptide portion is removed by specific protease cleavage. As specifically exemplified herein, a soluble, secreted GlcNAc T-V was engineered from the rat cDNA clone encoding GlcNAc T-V as described in U.S. Pat. 5,032,519 (Paulson et al., issued Jul. 16, 1991) with removal of the N-terminal 69 amino acids of rat GlcNAc T-V (see Example 14 for description of cloning). The DNA encoding the remainder of GlcNAc T-V was fused to the human gamma-interferon signal sequence coding region, and there is a Gln residue derived from the gamma-interferon at the N-terminus of the soluble GlcNAc T-V. The ordinary skilled artisan can readily produce soluble GlcNAc T-V derivations using the sequences provided herein, taken with what is well known to the art. Spent medium from cells expressing the soluble rat GlcNAc T-V was

chromatographed over a copper chelating column and over CM fast flow Sepharose to yield purified soluble GlcNAc T-V. Table 3 summarizes the results of soluble GlcNAc T-V purification as described in Example 15 herein. It was determined that there were protein bands of 95, 75 and 60 kDa which appeared to have enzymatic activity, although the 60 kDa band appeared to be less active. When EDTA (5 mM) is incorporated in the CM Sepharose column step, nearly all the protein is of about 95 kDa. Alternatively, a cocktail of protease inhibitors for maximizing the amount of 95 kDa protein can be added to the culture medium, removed for the copper chelation column, and provided again before the CM Sepharose cation exchange chromatography step. When no EDTA is used in the second column purification step, the predominant protein band revealed by SDS-PAGE is about 60 kDa, with minor bands at around 75 and 95 kDa. The N-terminal amino acid sequence analysis of the 60 kDa protein (purified by FPLC, gel filtration) is consistent with proteolytic cleavage between amino acids 283 and 284 of SEQ ID NO:16.

Detailed Description Text - DETX (103):
Isolation of PCR Fragment Containing Rat GlcNAc T-V Sequences

Detailed Description Text - DETX (156):
The cDNA libraries are screened for sequences encoding GlcNAc T-V by plaque hybridization under low stringency conditions using the approximately 200 bp amplimer radiolabelled by random hexamer labelling as described in Sambrook et al. (eds.) (1989) supra. Clones specifically hybridizing the amplimer sequence are selected for further analysis (restriction endonuclease digestion, nucleotide sequence determination).

Detailed Description Text - DETX (158):
From the clones analyzed it is possible to reconstruct the entire coding sequence of GlcNAc T-V. If a full-length coding sequence is not reconstructed, further primers can be designed using sequences near the ends of the sequenced region for use in the RACE procedure (Rapid Amplification of cDNA Ends) as described in Frohman et al. (1988) Proc. Natl. Acad. Sci. USA 85:8998-9002. Where the entire gene is desired, genomic libraries can be screened, and "walking" procedures known in the art are used to extend in both directions.

Detailed Description Text - DETX (160):
Cloning of a Rat cDNA Sequence Encoding GlcNAc T-V

Detailed Description Text - DETX (186):
SEQ ID NO:16 thus provides the primary structure (amino acid sequence) of rat GlcNAc T-V as including 740 specified amino acid residues (estimated M.W.=84,561 without glycosylation).

Detailed Description Text - DETX (197):
Isolation of Partial Mouse Sequences for GlcNAc T-V

Detailed Description Text - DETX (220):
An alternate approach to demonstrate that the full-length cDNA clone isolated does encode GlcNAc T-V, the coding sequence is fused to the N-terminal Protein A coding sequence as described in Larsen et al. (1989) Proc. Natl. Acad. Sci. USA 86:8227-8231. The resultant recombinant plasmid is then introduced into mammalian cells such that cells which have incorporated the cDNA sequences survive in culture. Because the fusion protein contains the N-terminal sequences of Protein .lambda., the fusion protein is directed to the secretion pathway and released from the cells. After removal of the cells by centrifugation, the culture medium is assayed for GlcNAc T-V activity as described herein. A portion of the cell-free medium is chromatographed over an IgG column to which the N-terminal Protein A sequences bind, causing GlcNAc T-V

activity to be retained on the column.

Detailed Description Text - DETX (221):
A second alternative approach for confirming that the cDNA isolated does encode GlcNAc T-V is to insert the complete cDNA into a vector under the control of regulatory sequences which will allow expression in the chosen mammalian host cells. The host cell chosen is a GlcNAc T-V-deficient variant of the mouse lymphoma BW5147 cell line, which variant is PHA 2.1; this variant cell line is described in Cummings et al. (1982) J. Biol. Chem. 257:13421-13427. An alternative GlcNAc T-V-deficient cell line is the Lec4 variant of CHO cells, described by Stanley, P. (1983) Methods Enzymol. 96:157-184. Both variant cells lines were selected for growth in the presence of the cytotoxic lectin L-phytohemagglutinin, which binds to the galactosylated product of GlcNAc T-V. Expression of the cDNA sequences encoding the GlcNAc T-V restores GlcNAc T-V activity and lectin sensitivity to these variant cell lines.

Detailed Description Text - DETX (224):
Determination of CHO GlcNAc T-V Sequence

Detailed Description Text - DETX (225):
The sequences for CHO GlcNAc T-V was readily determined using standard molecular biological techniques and the rat sequence information provided herein.

Detailed Description Text - DETX (229):
The CHO cDNA library was screened with a 5'342 bp rat GlcNAc T-V PCR amplimer which hybridizes 14 bp upstream from the ATG start codon and extends 328 nucleotides into the GlcNAc T-V coding region. Positive phage clones were then screened with a 3'320 bp rat GlcNAc T-V PCR amplimer which hybridizes 5 bp upstream from the TAG stop codon and extends into the 3' untranslated region. One putative CHO GlcNAc T-V clone hybridized to both the 5' and 3' rat GlcNAc T-V probes. Sequence analysis of the positive clone after second and third round plaque purification revealed the full length CHO GlcNAc T-V cDNA sequence (FIG. 11A-11F, SEQ ID NO:17)

Detailed Description Text - DETX (232):
Determination of Human GlcNAc T-V Sequence

Detailed Description Text - DETX (233):
The sequences for human GlcNAc T-V was readily determined using standard molecular biology procedures and the rat GlcNAc T-V sequence information provided herein.

Detailed Description Text - DETX (238):
The human GlcNAc T-V sequence upstream from the kidney PCR product was obtained by amplifying sequences in a commercially available human placenta cDNA library (Stratagene, #936203). Specific oligonucleotide primers from the human kidney PCR sequence were used as the 3' end antisense primers and a primer covering the T7 promoter sequence of the library cloning vector .lambda.ZAPII (Stratagene) was used as the 5' end sense primer. PCR was carried out using the following primers:

Detailed Description Text - DETX (243):
A comparison of the rat GlcNAc T-V sequence with the human sequence obtained by PCR of the human placenta cDNA library revealed that the human sequence lacked 14 bp from the ATG initiation codon. In order to obtain the extreme 5' end of the human GlcNAc T-V sequence, PCR was carried out using a sense primer designed to hybridize to sequences obtained from a portion of human GlcNAc T-V

genomic DNA. The 5' primer sequence was as follows:

Detailed Description Text - DETX (247):
The human GlcNAc T-V sequence downstream from the approximately 1125 bp kidney PCR product was obtained by amplifying sequences in the human placenta cDNA library using a specific oligonucleotide primer from the human kidney PCR sequence as the 5' end sense primer and a primer covering the T3 promoter sequence of the library cloning vector .lambda.ZAPII (Stratagene) as the 3' end antisense primer. PCR was carried out using the following primers:

Detailed Description Text - DETX (254):
Soluble, secreted recombinant rat GlcNAc T-V with enzymatic activity was produced by the methods described in U.S. Pat. No. 5,032,519, "Method for Producing Secretable Glycosyltransferases and Other Golgi Processing Enzymes," J. Paulson et al., Jul. 16, 1991. Briefly, the membrane anchor domain and the Golgi apparatus retention signal are deleted and the sequence information for expressing a cleavable secretion signal are inserted in the GlcNAc T-V genetic material. After transfection of the modified GlcNAc T-V sequences into cells, the cells secrete into the culture media soluble enzymatically active GlcNAc T-V. The GlcNAc T-V can be readily purified from the culture media for further use.

Detailed Description Text - DETX (255):
Using standard procedures and following the teachings of the cited patent, the cleavable signal sequence of human gamma-interferon was fused with the rat GlcNAc T-V at the sequence corresponding to amino acid number 70 of SEQ ID NO:16. This chimera has replaced the GlcNAc T-V putative cytoplasmic domain (amino acids 1-13), transmembrane domain (amino acids 14-30) and a portion of the stem region (amino acids 31-69) with a fragment coding for the 23 amino acid signal peptide and first amino acid of mature human gamma-interferon. The resulting fusion gene product is cleaved to yield secretable GlcNAc T-V containing one amino acid from the gamma-interferon (Gln) at the new NH.sub.2 -terminus.

Detailed Description Text - DETX (259):
Primer 553-28 (SEQ ID NO:33) contains the gamma-interferon signal sequence, one amino acid of the mature gamma-interferon and joins into the rat GlcNAc T-V sequence at amino acid 70. Primer 516-5 (SEQ ID NO:34) contains the rat GlcNAc T-V carboxy-terminal sequence and the TAG stop codon. After 20 cycles, the PCR products were cleaned using a PCR Magic Prep Kit (Promega Corporation) and a portion of the resulting sample was digested with Xbal and Sall. The restriction endonuclease digested sample was analyzed by agarose gel electrophoresis, and the approximately 2.1 kb PCR product was excised. The DNA was isolated using an S&S Elu-Quick DNA Purification Kit (Schleicher & Schuell) and ligated to an Xbal/Sall cut mammalian expression vector overnight at 16.degree. C. using T4 DNA ligase (Boehringher Mannheim, Indianapolis, Ind.). A portion of the ligation mixture was electroporated into E. coli DH10B cells as described in Example 7, Part A. Plasmid DNA that contained the correct insert was isolated from eight bacterial colonies using a Plasmid Midi Kit (Qiagen Inc., Chatsworth, Calif.).

Detailed Description Text - DETX (267):
Clone D was chosen for further analysis because it gave the highest enzyme activity in the radiochemical assay. The portion of the mammalian expression vector that carried the PCR derived insert was sequenced in both directions as described in Example 7, Part F. For Clone D, the human gamma-interferon signal sequence joined to the truncated rat GlcNAc T-V sequence was found to be 100% identical to the expected sequence.

Detailed Description Text - DETX (269):
The secreted rat GlcNAc T-V expression vector, clone D from above, was transfected into CHO dhfr.sup.- cells by the calcium phosphate precipitation method (Graham and van der Eb, Virology (1973) 52:456-467) modified as described by Wigler et al. (Cell (1978) 41:725-731) and Lewis et al. (Somatic Cell Genetics (1980) 6:333-347). Following selection by growth in media containing 5% dialyzed FBS (Irvine Scientific), pools and clones of stably transfected CHO dhfr.sup.- cells were obtained. Cell conditioned media from the transfected CHO dhfr.sup.- cell lines were collected and analyzed by the radionucleotide assay as described in Part B. The CHO dhfr.sup.- cell line which produced the highest amount of active soluble GlcNAc T-V as determined by the radiochemical assay (709 pmol/mg.times.hr) was used to seed a spinner cell culture flask. The cells were propagated in suspension cell culture and then used to seed roller bottles at an initial seeding density of 2.5.times.10.sup.7 cells in 200 ml of a 50/50 mixture of DMEM and F-12 media (Gibco) supplemented with 5% dialyzed FBS, 1.times. non-essential amino acids (Gibco) and 2 mM L-glutamine (Gibco). After three days the roller bottles were shifted to 200 ml of serum-free medium. Harvests were collected at 6-day intervals with new serum-free medium added after each harvest. In total, 62 liters of conditioned medium were harvested and concentrated to 2.4 liters by cross-flow ultrafiltration through Mini Sartocon polysulfone modules (Sartorius Corporation, Bohemia, N.Y.) then stored at -80.degree. C. prior to purification. Radionucleotide assays were carried out as described in Example 3 to analyze the GlcNAc T-V activity in the concentrated conditioned medium; the results demonstrated that approximately 26 units of total enzyme activity were produced.

Claims Text - CLTX (1):
1. A non-naturally occurring DNA molecule comprising a nucleotide sequence encoding a polypeptide having N-acetylglucosaminyl transferase V (GlcNAc T-V) activity.

Claims Text - CLTX (2):
2. The DNA molecule of claim 1 wherein said sequence encodes a mammalian GlcNAc T-V.

Claims Text - CLTX (3):
3. The DNA molecule of claim 2 wherein said nucleotide sequence encodes human GlcNAc T-V.

Claims Text - CLTX (6):
6. The DNA molecule of claim 2 wherein said nucleotide sequence encodes hamster GlcNAc T-V.

Claims Text - CLTX (11):
11. The recombinant DNA molecule of claim 10 wherein said nucleotide sequence encoding a polypeptide having N-acetylglucosaminyl transferase activity is selected from the group consisting of SEQ ID NO:17 from nucleotide 145 to nucleotide 2367 and SEQ ID NO:19 from nucleotide 38 to nucleotide 2263.

Claims Text - CLTX (17):
17. The recombinant cell of claim 15 wherein said nucleotide sequence encodes human GlcNAc T-V.

Claims Text - CLTX (18):
18. The recombinant cell of claim 17, wherein said nucleotide sequence encodes GlcNAc T-V having an amino acid sequence as given in SEQ ID NO:20.

Claims Text - CLTX (20):

20. The recombinant cell of claim 15 wherein said nucleotide sequence encodes hamster GlcNAc T-V.

Claims Text - CLTX (21):
21. The recombinant cell of claim 20, wherein said nucleotide sequence encodes GlcNAc T-V having an amino acid sequence as given in SEQ ID NO:18.

Claims Text - CLTX (24):
(a) operably linking a nucleotide sequence encoding a polypeptide having GlcNAc T-V activity to an expression control sequence to form a GlcNAc T-V expression cassette;

Claims Text - CLTX (26):
(c) culturing the GlcNAc T-V recombinant cell of step (b) under conditions appropriate for expression of said GlcNAc T-V expression cassette, whereby said nucleotide sequence directs expression of a polypeptide having GlcNAc T-V activity.

Claims Text - CLTX (27):
24. The method of claim 23 wherein said nucleotide sequence encodes a mammalian GlcNAc T-V.

Claims Text - CLTX (28):
25. The method of claim 24 wherein said nucleotide sequence encodes human GlcNAc T-V.

Claims Text - CLTX (31):
28. The method of claim 24 wherein said nucleotide sequence encodes hamster GlcNAc T-V.

Claims Text - CLTX (36):
(b) a portion of said non-naturally occurring DNA molecule having a DNA sequence capable of hybridizing to a DNA sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19, and which sequence encodes a polypeptide having GlcNAc T-V activity, or

Claims Text - CLTX (37):
(c) a portion of said non-naturally occurring DNA molecule having a DNA sequence encoding a soluble GlcNAc T-V.

US-PAT-NO: 5707846

DOCUMENT-IDENTIFIER: US 5707846 A

TITLE: N-acetylglucosaminyl transferase gene coding therefor and process for production thereof

DATE-ISSUED: January 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Taniguchi; Naoyuki	Toyonaka	N/A	N/A	JP
Nishikawa; Atsushi	Toyonaka	N/A	N/A	JP
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APPL-NO: 08/ 405230

DATE FILED: March 16, 1995

PARENT-CASE:

This application is a divisional of application Ser. No. 08/110,736, filed Aug. 23, 1993.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	4-245950	August 24, 1992
JP	5-237118	August 6, 1993

US-CL-CURRENT: 435/193

ABSTRACT:

A .beta.1,6-N-acetylglucosaminyl transferase having the following properties:

(1) Action: it transfers N-acetylglucosamine from UDP-N-acetylglucosamine to .alpha.-6-D-mannoside;

(2) Substrate specificity: it shows a reactivity of about 79% for GnGnF-bi-PA, about 125% for GnGnGn-tri-PA and about 66% for GnM-Pa, when taking a reactivity for GnGn-bi-PA as 100%;

(3) Optimum pH: 6.2 to 6.3;

(4) Inhibition, Activation and Stability: Mn.sup.2+ is not necessary for expression of activity, and the activity is not inhibited in the presence of 20 mM EDTA;

(5) Molecular weight: about 73,000 as determined by SDS-PAGE in the absence of reducing agent; and about 73,000 and about 60,000 as determined in the presence of a reducing agent;

(6) Km value: 133 .mu.M and 3.5 mM for acceptor GnGn-bi-PA and donor UDP-GlcNAc, respectively; and

(7) It includes the following peptide fragments:

(SEQ ID NO.1) Thr-Pro-Trp-Gly-Lys

(SEQ ID NO.2) Asn-Ile-Pro-Ser-Tyr-Val

(SEQ ID NO.3)
Val-Leu-Asp-Ser-Phe-Gly-Thr-Glu-Pro-Glu-Phe-Asn-His-Ala-Asn-Tyr-Ala

(SEQ ID NO.4) Asp-Leu-Gln-Phe-Leu-Leu

(SEQ ID NO.5) Asn-Thr-Asp-Phe-Phe-Ile-Gly,

and gene coding for said enzyme, and a process for production of the enzyme.

10 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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TITLE - TI (1):
N-acetylglucosaminyl transferase gene coding therefor and process for production thereof

Brief Summary Text - BSTX (3):
The present invention relates to an enzyme which transfers N-acetylglucosamine from UDP-N-acetylglucosamine to .alpha.-6-D-mannoside (UDP-N-Acetylglucosamine: .alpha.-6-D-Mannoside .beta.1-6N-Acetylglucosaminyl transferase; N-Acetylglucosaminyltransferase; hereinafter abbreviated as GnT-V), a gene coding therefor, and a process for production of GnT-V.

Brief Summary Text - BSTX (30):
The present enzyme .beta.1,6-N-acetylglucosaminyl transferase comprises, for example, an amino acid sequence comprising the amino acid sequence shown in SEQ ID No: 8, or an amino acid sequence wherein one or more than one amino acid residue is modified in the amino acid sequence shown in SEQ ID No: 8. Herein, the amino acid modification means that one or more amino acid residues are added, deleted and/or replaced with other amino acids.

Claims Text - CLTX (9):
2. An isolated .beta.1,6-N-acetylglucosaminyl transferase encoded by the nucleotide sequence of SEQ ID No: 8.

Claims Text - CLTX (10):
3. An isolated .beta.1,6-N-acetylglucosaminyl transferase having a nucleotide sequence having one or more nucleotide modifications in the nucleotide sequence of SEQ ID No: 8.

Claims Text - CLTX (11):
4. The isolated .beta.1,6-N-acetylglucosaminyl transferase according to claim 3 wherein the modification is an addition, deletion, replacement with other nucleotides, or a combination thereof.

US-PAT-NO: 5624832

DOCUMENT-IDENTIFIER: US 5624832 A
See image for Certificate of Correction

TITLE: .beta.1 6 N-acetylglucosaminyltransferase, its acceptor molecule, leukosialin, and a method for cloning proteins having enzymatic activity

DATE-ISSUED: April 29, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fukuda; Minoru	San Diego	CA	N/A	N/A
Bierhuizen; Marti F. A.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 227455

DATE FILED: April 14, 1994

PARENT-CASE:

This application is a divisional of application Ser. No. 07/995,041, filed Oct. 1, 1992, now U.S. Pat. No. 5,360,733.

US-CL-CURRENT: 435/193, 435/252.3 , 435/254.11 , 435/320.1 , 435/325 , 435/348 , 435/358 , 435/361 , 536/23.2

ABSTRACT:

The present invention provides a novel .beta.1.fwdarw.6 N-acetylglucosaminyltransferase, which forms core 2 oligosaccharide structures in O-glycans, and a novel acceptor molecule, leukosialin, CD43, for core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase activity. The amino acid sequences and nucleic acid sequences encoding these molecules, as well as active fragments thereof, also are disclosed. A method for isolating nucleic acid sequences encoding proteins having enzymatic activity is disclosed, using CHO cells that support replication of plasmid vectors having a polyoma virus origin of replication. A method to obtain a suitable cell line that expresses an acceptor molecule also is disclosed.

9 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Brief Summary Text - BSTX (13):
Thus, a need exists for identifying the core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase and the DNA sequences encoding this enzyme. The present invention satisfies this need and provides related advantages as well.

Drawing Description Text - DRTX (19):

An enzyme similar to the disclosed human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase has been purified from bovine tracheal epithelium (Ropp et al., J. Biol. Chem. 266:23863-23871 (1991), which is incorporated herein by reference. The apparent molecular weight of the bovine enzyme is .about.69 kDa. In comparison,, the predicted molecular weight of the polypeptide portion of core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase is .about.50 kDa. The deduced amino acid sequence of core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase reveals two to three potential N-glycosylation sites, suggesting N-glycosylation and O-glycosylation, or other post-translational modification, could account for the larger apparent size of the bovine enzyme.

Detailed Description Text - DETX (33):

No matches were obtained when the C2GnT cDNA sequence and deduced amino acid sequence were compared with sequences listed in the PC/Gene 6.6 data bank. In particular, no homology was revealed between the deduced amino acid sequence of C2GnT and other glycosyltransferases, including N-acetylglucosaminyltransferase I (Sarkar et al., Proc. Natl. Acad. Sci. USA 88:234-238 (1991), which is incorporated herein by reference).

Other Reference Publication - OREF (14):

Kawashima, Hiroto, et al., "Purification and Characterization of UDP-GlcNAc:Gal.beta.1-4Glc (NAc) .beta.-1, 3-N-Acetylglucosaminyltransferase (Poly-N-acetyllactosamine Extension Enzyme) from Calf Serum." J. Biol. Chem. 268:27118-27126 (1993).

US-PAT-NO: 5501957

DOCUMENT-IDENTIFIER: US 5501957 A

TITLE: Method for measuring glycosyltransferase activity

DATE-ISSUED: March 26, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dennis; James W.	Etobicoike	N/A	N/A	CA
Siminovitch; Katherine A.	Toronto	N/A	N/A	CA
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APPL-NO: 08/ 293940

DATE FILED: August 22, 1994

PARENT-CASE:

This application is a continuation of Ser. No. 07/968,865, filed Oct. 30, 1992, now abandoned.

US-CL-CURRENT: 435/15, 435/14 , 435/4 , 435/810 , 435/97 , 436/92 , 436/94 , 514/1 , 514/23 , 514/54 , 536/1.11 , 536/29.11

ABSTRACT:

A method of assaying for glycosyltransferase activity in a sample. In a first step, a sample is reacted with a first sugar donor and an acceptor substrate to produce a transferase product. The first sugar donor and acceptor substrate are selected such that the sugar from the first sugar donor is capable of being transferred to the acceptor substrate in the presence of the glycosyltransferase to be assayed. In a second step, the transferase product is reacted with a second sugar donor having a sugar which is labelled with a labelling agent and an enzyme which is capable of transferring the sugar from the second sugar donor to the transferase product to produce a labelled transferase product and which has a higher affinity for the glycosyltransferase product compared to the affinity of the glycosyltransferase for the acceptor substrate. The labelling agent activity of the labelled transferase product or unreacted second sugar donor is assayed to determine transferase activity in the sample. A kit for assaying for glycosyltransferase activity in a sample is also described.

28 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

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Brief Summary Text - BSTX (18):

The method of the invention may be used to assay for glucosaminyltransferases including the following: UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R

.beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosaminyltransferase. The method of the invention may also be used to assay for glucosyltransferases.

Brief Summary Text - BSTX (22):

The kit may be used to assay for glucosaminyltransferases such as UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosaminyltransferase. The kit may also be used to assay for glucosyltransferases.

Detailed Description Text - DETX (85):

Elevated cAMP levels alone, were not sufficient to induce core 2 GlcNAc-T activity, since cholera toxin (Table 3), dibutyryl-cAMP and 8-bromo-cAMP failed to induce enzyme activity. It is possible that in CHO cells induction of core 2 GlcNAc-T by butyrate requires gene transcription, followed by cAMP-dependent protein phosphorylation. In this regard, the time course profile for butyrate induced increases in cAMP was strikingly similar to that of core 2 GlcNAc-T induction; with a first peak at 12 hours and a second at 72 hours (FIG. 10A). Furthermore, raising intracellular cAMP levels in butyrate-treated CHO cells by the addition of cholera toxin (see FIG. 10B), enhanced core 2 GlcNAc-T activity by 80%, from 130 to 223 pmoles/mg/h at 24 h (FIG. 11). FIG. 11 shows a time-course comparison of core 2 GlcNAc-T activity in butyrate, cholera toxin, and butyrate+cholera toxin treated CHO cells. Cell cultures were grown in the presence of 2 mM butyrate (), 2 mM Na-butyrate+100 ng/ml cholera toxin (), or 100 ng/ml cholera toxin () for up to 72 hours. Cells were harvested at various time points and enzyme activity was measured as described above. Similar increases in core 2 GlcNAc-T expression were observed when 1.5 mM dibutyryl cAMP and 1.5 mM 8-bromo-cAMP were added to butyrate-treated cells.

Detailed Description Text - DETX (99):

A comparison of the kinetic properties of core 2 GlcNAc-T revealed that the Km for sugar nucleotide substrate has increased approximately 9 fold in butyrate+cholera toxin-treated cells compared to untreated CHO cells. However, no changes in Km for the synthetic acceptor were observed. Despite the high Km for UDP-GlcNAc, Vmax for core 2 GlcNAc-T in treated cells increased 80 fold with respect to untreated cells. Expressed as Vmax/Km, the relative catalytic activity of the enzyme was 8.8 fold greater in treated cells compared to untreated cells. A decrease in the affinity of core 2 GlcNAc-T for UDP-GlcNAc may be due to a post-translational modification of the enzyme such as phosphorylation, or at the gene level, expression of a second core 2 GlcNAc-T gene, or alternate splicing of a single gene transcript. It is possible that acceptors for core 2 GlcNAc-T also affect the sugarnucleotide Km for core 2 GlcNAc-T, which would imply an altered acceptor preference for the enzyme following butyrate treatment in CHO cells.

Claims Text - CLTX (12):
10. A method as claimed in claim 9 wherein the glucosaminyltransferase to be assayed is UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosamimyltransferase.

Claims Text - CLTX (24):
22. A kit as claimed in claim 21 wherein the glucosaminyltransferase to be assayed is UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosaminyltransferase.

PGPUB-DOCUMENT-NUMBER: 20040018590

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018590 A1

TITLE: Combinatorial DNA library for producing modified N-glycans in lower eukaryotes

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

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Nett, Juergen Hermann	Grantham	NH	US	
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APPL-NO: 10/ 371877

DATE FILED: February 20, 2003

RELATED-US-APPL-DATA:

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non-provisional-of-provisional 60214358 20000628 US

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non-provisional-of-provisional 60279997 20010330 US

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 530/395 , 536/23.2 , 536/53

ABSTRACT:

The present invention relates to eukaryotic host cells having modified oligosaccharides which may be modified further by heterologous expression of a set of glycosyltransferases, sugar transporters and mannosidases to become host-strains for the production of mammalian, e.g., human therapeutic glycoproteins. The invention provides nucleic acid molecules and combinatorial libraries which can be used to successfully target and express mammalian enzymatic activities such as those involved in glycosylation to intracellular compartments in a eukaryotic host cell. The process provides an engineered host cell which can be used to express and target any desirable gene(s) involved in glycosylation. Host cells with modified oligosaccharides are created or selected. N-glycans made in the engineered host cells have a Man.sub.5GlcNAc.sub.2 core structure which may then be modified further by heterologous expression of one or more enzymes, e.g., glycosyltransferases, sugar transporters and mannosidases, to yield human-like glycoproteins. For the production of therapeutic proteins, this method may be adapted to engineer cell lines in which any desired glycosylation structure may be obtained.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/892,591, filed Jun. 27, 2001, in which priority is claimed to U.S. Provisional Application Serial No. 60/214,358, filed Jun. 28, 2000, U.S. Provisional Application No. 60/215,638, filed Jun. 30, 2000, and U.S. Provisional Application No. 60/279,997, filed Mar. 30, 2001; each of which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (30):
[0024] Japanese Patent Application Publication No. 8-336387 discloses the deletion of an OCHI homolog in Pichia pastoris. In S. cerevisiae, OCHI encodes a 1,6-mannosyltransferase, which adds a mannose to the glycan structure Man.sub.8GlcNAc.sub.2 to yield Man.sub.9GlcNAc.sub.2. The Man.sub.9GlcNAc.sub.2 structure, which contains three 1,6 mannose residues, is then a substrate for further 1,2-, 1,6-, and 1,3- mannosyltransferases in vivo, leading to the hypermannosylated glycoproteins that are characteristic for S. cerevisiae and which typically may have 30-40 mannose residues per N-glycan. Because the Och1p initiates the transfer of 1,6 mannose to the Man.sub.8GlcNAc.sub.2 core, it is often referred to as the "initiating 1,6 mannosyltransferase" to distinguish it from other 1,6 mannosyltransferases acting later in the Golgi. In an och1 mnn1 mnn4 mutant strain of S. cerevisiae, proteins glycosylated with Man.sub.8GlcNAc.sub.2 accumulate and hypermannosylation does not occur. However, Man.sub.8GlcNAc.sub.2 is not a substrate for mammalian glycosyltransferases, such as human UDP-GlcNAc transferase I, and accordingly, the use of that mutant strain, in itself, is not useful for producing mammalian-like proteins, i.e., with complex or hybrid glycosylation patterns.

Detail Description Paragraph - DETX (57):
[0116] Accordingly, some or all of the Man.sub.5GlcNAc.sub.2 produced by the selected host cell must be a productive substrate for enzyme activities along a mammalian glycosylation pathway, e.g., can serve as a substrate for a GlcNAc transferase I activity in vivo, thereby forming the human-like N-glycan intermediate GlcNAcMan.sub.5GlcNAc.sub.2 in the host cell. In a preferred embodiment, at least 10%, more preferably at least 30% and most preferably 50% or more of the Man.sub.5GlcNAc.sub.2 intermediate produced in the host cell of the invention is a productive substrate for GnTI in vivo. It is understood that if, for example, GlcNAcMan.sub.5GlcNAc.sub.2 is produced at 10% and Man.sub.5GlcNAc.sub.2 is produced at 25% on a target protein, that the total amount of transiently produced Man.sub.5GlcNAc.sub.2 is 35% because GlcNAcMan.sub.5GlcNAc.sub.2 is a product of Man.sub.5GlcNAc.sub.2.

Detail Description Paragraph - DETX (78):
[0134] Lower eukaryotes that are able to produce glycoproteins having the attached N-glycan Man.sub.5GlcNAc.sub.2 are particularly useful because (a) lacking a high degree of mannosylation (e.g. greater than 8 mannoses per N-glycan, or especially 30-40 mannoses), they show reduced immunogenicity in humans; and (b) the N-glycan is a substrate for further glycosylation reactions to form an even more human-like glycoform, e.g., by the action of GlcNAc transferase I (FIG. 1B; .beta.1,2 GnTI) to form GlcNAcMan.sub.5GlcNAc.sub.2. A yield is obtained of greater than 30 mole %, more preferably a yield of 50-100 mole %, glycoproteins with N-glycans having a Man.sub.5GlcNAc.sub.2 structure. In a preferred embodiment, more than 50% of the Man.sub.5GlcNAc.sub.2 structure is shown to be a substrate for a GnTI activity and can serve as such a substrate in vivo.

Detail Description Paragraph - DETX (187):
[0240] Examples of modifications to glycosylation which can be affected using a method according to this embodiment of the invention are: (1) engineering a eukaryotic host cell to trim mannose residues from Man.sub.8GlcNAc.sub.2 to yield a Man.sub.5GlcNAc.sub.2 N-glycan; (2) engineering eukaryotic host cell to add an N-acetylglucosamine (GlcNAc) residue to Man.sub.5GlcNAc.sub.2 by action of GlcNAc transferase I; (3) engineering a eukaryotic host cell to functionally express an enzyme such as an N-acetylglucosaminyl Transferase (GnTI, GnTII, GnTIII, GnTIV, GnTV, GnTVI), mannosidase II, fucosyltransferase (FT), galactosyl tranferase (GalT) or a sialyltransferase (ST).

Detail Description Paragraph - DETX (292):
[0314] A portion of the gene encoding human N-acetylglucosaminyl Transferase I (MGATI, Accession# NM002406), lacking the first 154 bp, was amplified by PCR using oligonucleotides 5'-TGGCAGGCGCGCCTCAGTCAGCGCTCTCG-- 3' (SEQ ID NO:32) and 5'-AGGTTAATTA AGTGCTAATTCCAGCTAGG-3' (SEQ ID NO:33) and vector pHG4.5 (ATCC# 79003) as template. The resulting PCR product was cloned into pCR2.1-TOPO and the correct sequence was confirmed. Following digestion with AscI and PacI the truncated GnTI was inserted into plasmid pJN346 to create pNA. After digestion of pJN271 with NotI and AscI, the 120 bp insert was ligated into pNA to generate an in-frame fusion of the MNN9 transmembrane domain with the GnTI, creating pNA 15.

PGPUB-DOCUMENT-NUMBER: 20020137134

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137134 A1

TITLE: Methods for producing modified glycoproteins

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 892591

DATE FILED: June 27, 2001

RELATED-US-APPL-DATA:

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non-provisional-of-provisional 60215638 20000630 US

non-provisional-of-provisional 60279997 20010330 US

US-CL-CURRENT: 435/69.1, 435/200 , 435/254.23 , 435/320.1 , 530/395

ABSTRACT:

Cell lines having genetically modified glycosylation pathways that allow them to carry out a sequence of enzymatic reactions, which mimic the processing of glycoproteins in humans, have been developed. Recombinant proteins expressed in these engineered hosts yield glycoproteins more similar, if not substantially identical, to their human counterparts. Thelower eukaryotes, which ordinarily produce high-mannose containing N-glycans, including unicellular and multicellular fungi are modified to produce N-glycans such as Man.sub.5GlcNAc.sub.2 or other structures along human glycosylation pathways. This is achieved using a combination of engineering and/or selection of strains which: do not express certain enzymes which create the undesirable complex structures characteristic of the fungal glycoproteins, which express exogenous enzymes selected either to have optimal activity under the conditions present in the fungi where activity is desired, or which are targeted to an organelle where optimal activity is achieved, and combinations thereof wherein the genetically engineered eukaryote expresses multiple exogenous enzymes required to produce "human-like" glycoproteins.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] Priority is claimed to U.S. Provisional Application Serial No. 60/214,358, filed on Jun. 28, 2000, U.S. Provisional Application Serial No. 60/215,638, filed on Jun. 30, 2000, and U.S. Provisional Application Serial No. 60/279,997, filed on Mar. 30, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (36):

[0035] In addition, Japanese Patent Application Public No. 8-336387 discloses an OCH1 mutant strain of Pichia pastoris. The OCH1 gene encodes 1,6-mannosyltransferase, which adds a mannose to the glycan structure Man.sub.8GlcNAc.sub.2 to yield Man.sub.9GlcNAc.sub.2. The Man.sub.9GlcNAc.sub.2 structure is then a substrate for further mannosylation in vivo, leading to the hypermannosylated glycoproteins that are characteristic of yeasts and typically may have at least 30-40 mannose residue per N-glycan. In the OCH1 mutant strain, proteins glycosylated with Man.sub.8GlcNAc.sub.2 are accumulated and hypermannosylation does not occur. However, the structure Man.sub.8GlcNAc.sub.2 is not a substrate for animal glycosylation enzymes, such as human UDP-GlcNAc transferase I, and accordingly the method is not useful for producing proteins with human glycosylation patterns.

Detail Description Paragraph - DETX (5):

[0050] Lower eukaryotes that are able to produce glycoproteins having the attached N-glycan Man.sub.5GlcNAc.sub.2 are particularly useful since (a) lacking a high degree of mannosylation (e.g. greater than 8 mannoses per N-glycan, or especially 30-40 mannoses), they show reduced immunogenicity in humans; and (b) the N-glycan is a substrate for further glycosylation reactions to form an even more human-like glycoform, e.g. by the action of GlcNAc transferase I to form GlcNAcMan.sub.5GlcNAc.sub.2. Man.sub.5GlcNAc.sub.2 must be formed in vivo in a high yield, at least transiently, since all subsequent glycosylation reactions require Man.sub.5GlcNAc.sub.2 or a derivative thereof. Accordingly, a yield is obtained of greater than 27 mole %, more preferably a yield of 50-100 mole %, glycoproteins in which a high proportion of N-glycans have Man.sub.5GlcNAc.sub.2. It is then possible to perform further glycosylation reactions in vitro, using for example the method of U.S. Pat. No. 5,834,251 to Maras and Contreras. In a preferred embodiment, at least one further glycosylation reaction is performed in vivo. In a highly preferred embodiment thereof, active forms of glycosylating enzymes are expressed in the endoplasmic reticulum and/or Golgi apparatus.

Detail Description Paragraph - DETX (37):

[0082] Examples of modifications to glycosylation which can be effected using method are: (1) engineering an eukaryotic microorganism to trim mannose residues from Man.sub.8GlcNAc.sub.2 to yield Man.sub.5GlcNAc.sub.2 as a protein N-glycan; (2) engineering an eukaryotic microorganism to add an N-acetylglucosamine (GlcNAc) residue to Man.sub.5GlcNAc.sub.2 by action of GlcNAc transferase I; (3) engineering an eukaryotic microorganism to functionally express an enzyme such as an N-acetylglucosamine transferase (GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI), mannosidase II, fucosyltransferase, galactosyl tranferase (GalT) or sialyltransferases (ST).

US-PAT-NO: 6131578

DOCUMENT-IDENTIFIER: US 6131578 A

TITLE: Inhibitors of UDP-G1cNAc:Ga1.beta.1-3Ga1NAc.alpha.R .beta.1-6 N-acetylglucosaminyltransferase (core 2 G1cNAc-T) and use of the inhibitors to prevent or treat cardiomyopathy associated with diabetes

DATE-ISSUED: October 17, 2000

INVENTOR-INFORMATION:

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APPL-NO: 08/ 943058

DATE FILED: October 2, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of provisional application 60/046,876 filed Oct. 2, 1996 now abandoned.

US-CL-CURRENT: 128/898, 514/44

ABSTRACT:

Cardiomyopathy associated with diabetes and hyperglycemia can be treated by administering to a subject suffering from this condition a substance that inhibits UDP-GlcNAc:Gal.beta.1-3GalNAc.alpha.R.beta.1-6-N-acetylglucosaminyl transferase (core 2 GlcNAc-T) activity.

10 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Drawing Description Text - DRTX (13):
FIG. 9 shows the nucleotide and amino acid sequence of human core 2 GlcNAc-T.

Detailed Description Text - DETX (24):
In accordance with one embodiment of the invention, the transgenic non-human animal contains a DNA construct comprising a gene encoding core 2 GlcNAc-T. In accordance with a preferred embodiment of the invention, the transgenic

non-human animal contains a DNA construct comprising a gene encoding core 2 GlcNAc-T and a promoter which stimulates expression of the gene in the cardiovascular system. Suitable promoters include the cardiac myosin promoter.

Detailed Description Text - DETX (37):

cDNA cloning of mouse UDP-GlcNAc:Gal.beta.1-3Gal/NAc-.varies.R .beta.1-6 N-acetylglucosaminyltransferase (core 2 GlcNAc-T). Approximately 2.times.10.sup.5 colonies of a cDNA library preapred in pCDM8 (Invitrogen Corp.) using poly A+RNA from D33W25, a murine lymphoid tumor cell line (22), were screened by colony hybridization (23) to a 864-bp EcoRI-BamH1 subcloned fragment of human core 2 GlcNAc-T isolated by PCR (gift of Dr. A. Datti, Perugia, Italy) corresponding to amino acids 85-372 of the human enzyme. Hybridization was performed overnight at 65.degree. C. in 500 mM sodium phosphate pH 7.2, 7% SDS, 1% BSA, 1 mM. EDTA After rinsing, filters were washed at 65.degree. C. in 100 mM sodium phosphate, 0.1% SDS. After three rounds of hybridization and purification, two clones, designated C2-251 and C2-352, were isolated and gave specific and strongly positive signals on Southern blots hybridized with the probe. The cDNA inserts were subcloned as XhoI fragments into SalI cut pGEM5zf(+) (Promega Corp., Madison, Wis.) and a series of exonuclease III-mung bean nuclease (GIBCO BRL)--nested deletions generated from each end. DNA sequencing was performed using the Autoread sequencing kit and the ALF DNA sequencer according to the manufacturer's instructions (Pharmacia LKB Biotechnology). Some sequences were also generated using internal primers. Data were analyzed and edited using the UWGCG suite.

Detailed Description Text - DETX (46):

Cloning full-length DH1 cDNA. To facilitate identification, a cDNA library derived from diabetic rat heart mRNA was screened using the 214-bp-cloned DH1 PCR fragment as a probe. Five overlapping recombinants were identified and the composite of the full cDNA sequence was determined (FIG. 3(A)). It contained 5,010 bp inclusive of poly A tail and corresponded to the size detected by the original Northern blot analysis. Open reading frame analysis showed that the longest possible coding region which was from position 802 to 2085 and encoded 428 amino acids. The GXXATGC pattern was observed flanking the region of the presumptive start codon (31) and a polyadenylation signal, AATAAA, was found 15 bp upstream from the polyA tail. Searches for homologous sequences in Genbank/EMBL revealed that this cDNA shared 80% identity at the nucleotide level and 85% identity at the amino acid level with human core 2 (GlcNAc-T) (32). The mouse core 2 GlcNAc-T was also cloned and sequenced and it was found that DH1 shared 92% identity with the amino acid sequence of mouse core 2 GlcNAc-T (FIG. 3(B)). These findings strongly suggested that DH1 was rat core 2 GlcNAc-T.

US-PAT-NO: 6015701

DOCUMENT-IDENTIFIER: US 6015701 A
See image for Certificate of Correction

TITLE: N-acetylglucosaminyltransferase V proteins and coding sequences

DATE-ISSUED: January 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Fregien; Nevis Lee	Miami	FL	N/A	N/A

APPL-NO: 08/ 276968

DATE FILED: July 19, 1994

PARENT-CASE:

This application is a continuation-in-part of U.S. Ser. No. 08/016,863, filed Feb. 10, 1993, now U.S. Pat. No. 5,602,003, which application is a continuation-in-part of U.S. Ser. No. 07/905,795, filed Jun. 29, 1992, now abandoned.

US-CL-CURRENT: 435/193, 435/252.3 , 435/252.33 , 435/320.1 , 435/325 , 536/23.2

ABSTRACT:

The present invention provides substantially purified UDP-N-acetylglucosamine: .alpha.-6-D-mannoside .beta.-1,6-N-acetylglucosaminyl transferase (GlcNAc T-V; EC 2.4.1.155) proteins and antibodies which specifically bind GlcNAc T-V. The present invention also provides polynucleotide sequences and oligonucleotide probes capable of specifically hybridizing to nucleic acid sequences which encode GlcNAc T-V, and cDNA and genomic clones encoding GlcNAc T-V, as well as nucleotide sequences encoding GlcNAc T-V, as specifically exemplified by GlcNAc T-V coding sequences from rat, hamster, mouse and human.

31 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 20

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Brief Summary Text - BSTX (2):
The field of this invention is the area of protein glycosylation, specifically the area of the particular enzyme, UDP N-acetylglucosaminyltransferase V, involved in the expression of the .beta.(1,6) branch structure found in tri- and tetraantennary N-linked

oligosaccharides. The field relates to purified active enzyme, the amino acid sequences of rat, human and hamster GlcNAc T-V proteins, genes encoding active enzyme and cell lines genetically engineered to express a nucleotide sequence encoding active enzyme.

Brief Summary Text - BSTX (16):
Also provided in this invention is GlcNAc T-V produced by recombinant DNA technology in prokaryotic or eukaryotic host cells. Disclosed in this invention are the complete amino acid sequences for rat, human and hamster (e.g., Chinese Hamster Ovary (CHO) cells) GlcNAc T-V and nucleotide sequences encoding rat, human and hamster GlcNAc T-V. Examples of methods of producing recombinant active GlcNAc T-V by recombinant DNA technology are disclosed. The exemplified amino acid sequences and the nucleotide sequences encoding GlcNAc T-V, and subsequences within, as understood in the art, will be useful for isolating GlcNAc T-V coding sequences from a wide range of species and for producing useful quantities of GlcNAc T-V by recombinant DNA technology.

Brief Summary Text - BSTX (17):
Further objects of this invention are cDNA clones encoding GlcNAc T-V and genomic clones encoding GlcNAc T-V. The antibodies raised against rat kidney GlcNAc T-V (or other GlcNAc T-V's or peptide-specific antibodies for GlcNAc T-V) can be used to detect expression of GlcNAc T-V from sources other than rat kidney by virtue of cross-reactivity with those other GlcNAc T-V enzymes; alternatively, these antibodies can be used to screen cDNA expression libraries. Sequences encoding GlcNAc T-V from rat, human and hamster (i.e., Chinese hamster ovary) cells and a partial coding sequence from mouse are presented herein. Similarly, the degenerate oligonucleotide probes and/or the coding sequence and/or the amplimer sequences of the present invention can be used to screen genomic or cDNA libraries constructed using nucleic acids from sources other than those exemplified herein, or these can be used to prepare primers to amplify sequences encoding GlcNAc T-V from mRNA populations prepared from rat kidney or from other animal cells. The cDNA and/or genomic sequences encoding GlcNAc T-V will be useful in directing the recombinant expression of GlcNAc T-V.

Brief Summary Text - BSTX (18):
Further objects of this invention are nucleotide sequences encoding rat GlcNAc T-V, and nucleotide sequences encoding GlcNAc T-V from other vertebrate, preferably mammalian, sources, including cDNA and genomic sequences. The nucleotide sequence encoding rat GlcNac T-V is provided herein as SEQ ID NO:15, from an ATG translation start codon beginning at nucleotide 299 through a translation stop codon ending at nucleotide 2521. The nucleotide sequence encoding human GlcNac T-V is provided herein as SEQ ID NO:19, from an ATG translation start codon beginning at nucleotide 38 through a translation stop codon ending at nucleotide 2263. The nucleotide sequence encoding hamster (i.e., CHO cells) GlcNac T-V is provided herein as SEQ ID NO:17, from an ATG translation start codon beginning at nucleotide 145 through a translation stop codon ending at nucleotide 2367. A partial mouse cDNA sequence is given in SEQ ID NO:21.

Brief Summary Text - BSTX (19):
The skilled artisan recognizes that there will be more than one nucleotide sequence capable of encoding the same amino acid sequence due to the degeneracy of the genetic code. Exemplary GlcNAc T-V amino acid sequences are given in SEQ ID NOs 16, 18 and 20. These sequences, and sequence variants thereof which encode functionally equivalent GlcNAc T-V, can be used to express GlcNAc T-V in a desired recombinant host cell. The GlcNAc T-V coding sequences from other vertebrate species, preferably from mammals, will be highly homologous at the nucleotide sequence level to the exemplified rat, hamster and human GlcNAc T-V

coding sequence disclosed herein. Functionally equivalent GlcNAc T-V coding sequences with at least 70%, preferably at least 80%, more preferably at least 90% nucleotide sequence homology to the exemplified rat, human and/or hamster (CHO) GlcNAc T-V coding sequences can be identified and isolated from cDNA libraries prepared from mRNA sources other than rat, human and CHO cells, using well-known DNA-DNA hybridization technology and the exemplified GlcNAc T-V coding sequences provided herein. Also contemplated are genomic clones encoding GlcNAc T-V, which clones comprise the natural regulatory sequences. It is understood that any intron sequences in genomic GlcNAc T-V are not to be included in sequence comparisons to the exemplified full-length coding sequence, and gaps may be introduced to maximize homology.

Brief Summary Text - BSTX (21):

Still further objects of the invention are cells genetically engineered to contain a DNA molecule containing a first nucleotide sequence encoding an enzymatically active GlcNAc T-V and a second nucleotide sequence not found associated with the GlcNAc T-V coding sequence in nature. Mammalian cells are preferred for recombinant expression of GlcNAc T-V coding sequences. Particularly preferred are COS-7 cells and CHO (Chinese Hamster Ovary) cells. The exemplified rat, CHO and human GlcNAc T-V amino acid sequences are particularly preferred, preferably encoded by the exemplified nucleotide coding sequences as in FIG. 11 or SEQ ID NO:15 from nucleotide 299 through nucleotide 2521, in SEQ ID NO:17 from nucleotide 145 through nucleotide 2367, and in SEQ ID NO:19 from nucleotide 38 through nucleotide 2263.

Drawing Description Text - DRTX (13):

FIG. 12A-12B, taken in sequence, presents a comparison of the deduced amino acid sequences of human (SEQ ID NO:20), CHO (SEQ ID NO:18), mouse (SEQ ID NO:22) and rat (SEQ ID NO:16) GlcNAc T-V. The human sequence contains an insertion of a valine at amino acid 109 as compared to the CHO and rat sequences. The mouse sequence represents the analysis of a partial cDNA clone starting at amino acid 288 according to the numbering in SEQ ID NO:16. The asterisks signify the end of the protein.

Detailed Description Text - DETX (18):

It is understood by those skilled in the art that the exemplified rat GlcNAc T-V coding sequence, provided herein in FIG. 10 and in SEQ ID NO:15 from nucleotide 299 through nucleotide 2521, is representative of GlcNAc T-V from other vertebrate sources, especially of other mammalian sources, including humans. SEQ ID NO:17 and SEQ ID NO:19 provide the CHO and human sequences, respectively, encoding GlcNAc T-V, and SEQ ID NO:21 provides a partial mouse sequence encoding GlcNAc T-V. The coding sequences for GlcNAc T-V provided herein are suitable for use in preparing or deriving PCR primers for identifying and/or amplifying sequences encoding human or other animal GlcNAc T-V, and/or for use as hybridization probes to identify clones encoding human, hamster, rat, other mammalian or other vertebrate GlcNAc T-V in appropriate genomic or cDNA libraries.

Detailed Description Text - DETX (19):

The techniques for the purification of the rat kidney GlcNAc T-V disclosed herein will be understood to be applicable to the purification of human or other GlcNAc T-V to a level comparable to that of rat kidney GlcNAc T-V. The skilled artisan recognizes that routine modifications of the procedures disclosed herein may provide improved results in isolating nonexemplified GlcNAc T-V enzymes.

Detailed Description Text - DETX (20):

Species other than rat, mouse, hamster and human contain genes encoding proteins which catalyze the same enzymatic reaction as rat GlcNAc T-V, which

genes have significant sequence homology to the rat, hamster, mouse and human sequences encoding GlcNAc T-V. One can isolate these homologous cDNAs and/or genes using the DNA sequences of this invention as probes or primers under standard hybridization conditions. This invention specifically contemplates and encompasses such sequences.

Detailed Description Text - DETX (21):

A comparison of the human, CHO, rat and partial mouse GlcNAc T-V nucleotide sequences is presented in FIGS. 11A-11F (SEQ ID NOS:19, 17, 15 and 21, respectively). The coding region of SEQ ID NO:15 extends from an ATG starting at nucleotide 299 to a stop codon ending at nucleotide 2524. The rat sequence contains 298 bp of upstream 5' untranslated sequence. The human and the CHO sequences contain 136 bp and 243 bp of 5' untranslated sequence respectively. The partial mouse sequence is presented starting within the coding region at the nucleotide numbered 1159 of SEQ ID NO:15. In addition, approximately 300 bp of the human, 100 bp of the rat and 325 bp of the mouse 3' untranslated regions are provided. Analysis of the coding regions of these sequences indicates that there is approximately 89% homology of the human sequence compared with the rat sequence. The CHO sequence shares an approximately 93% homology with the rat sequence. In a comparison of the partial mouse coding region with the corresponding portion of the rat sequence, approximately 96% nucleotide sequence homology is obtained.

Detailed Description Text - DETX (22):

In FIGS. 12A-12B the human (SEQ ID NO:20), CHO (SEQ ID NO:18), rat (SEQ ID NO:16) and partial mouse (SEQ ID NO:22) GlcNAc T-V deduced amino acid sequences are compared. The partial mouse sequence is presented starting at amino acid 288 of SEQ ID NO:16. The human GlcNAc T-V sequence contains an additional valine at amino acid 109 compared to the rat and CHO sequences. The available mouse sequence does not extend to this region. The additional amino acid in the human sequence occurs at the site of the first potential N-linked glycosylation site, although the potential glycosylation sequence is maintained. The human, CHO and rat sequences all contain the same six potential N-glycosylation sites. The mouse sequence also shares the three potential N-glycosylation sites that are located within the available GlcNAc T-V sequence. There is approximately 98% amino acid sequence identity between human and rat amino acid sequences. The CHO amino acid sequence is approximately 99% identical with the rat, and the mouse amino acid sequence is greater than 99% identical with the rat over the region for which the mouse sequence was obtained.

Detailed Description Text - DETX (23):

Thus, GlcNAc T-V coding sequences from vertebrate sources have significant sequence homology to the exemplified rat, human and hamster GlcNAc T-V coding sequences and the encoded GlcNAc T-V enzymes have a high degree of amino acid sequence identity as disclosed herein. It is obvious to one normally skilled in the art that human, rat and CHO GlcNAc T-V cDNA clones, genomic clones and PCR ampliners can be readily isolated using standard procedures and the sequence information provided herein. There would be no need to practice these examples exactly, but rather the sequence information provided herein (SEQ ID NOs 15-22) enables the isolation of rat, CHO, mouse, human and other GlcNAc T-V nucleic acid coding sequences and amino acid sequences. It is further obvious to one normally skilled in the art that, as demonstrated in Examples 12 and 13, GlcNAc T-V cDNA and genomic clones, cDNA and genomic gene sequences, and amino acid sequences can be readily obtained and used for GlcNAc T-V from any mammalian species using standard procedures and the sequence information provided herein. The ordinary skilled artisan can utilize the exemplified sequences provided herein, or portions thereof, preferably at least 25-30 bases in length, in hybridization probes to identify cDNA (or genomic) clones

encoding GlcNAc T-V, where there is at least 70% sequence homology to the probe sequence using appropriate art-known hybridization techniques. The skilled artisan understands that the capacity of a cloned cDNA to encode functional GlcNAc T-V enzyme can be readily tested as taught herein (See Example 11).

Detailed Description Text - DETX (61):

Soluble secreted GlcNAc T-V enzyme proteins can be produced using the disclosure provided herein. A soluble GlcNA T-V is one which lacks the sequences in the amino terminal region of the protein which localize it to and bind it within the cell membrane, particularly within the Golgi apparatus. When the coding region of the enzymatically active portion of GlcNAc T-V, but not including the transmembrane region, is fused downstream of and in frame with a signal sequence coding sequence, and operably linked to transcriptional control sequences, and expressed in a suitable host cell, such as a mammalian cell, soluble GlcNAc T-V is expressed and secreted into the culture medium after the signal peptide portion is removed by specific protease cleavage. As specifically exemplified herein, a soluble, secreted GlcNAc T-V was engineered from the rat cDNA clone encoding GlcNAc T-V as described in U.S. Pat. 5,032,519 (Paulson et al., issued Jul. 16, 1991) with removal of the N-terminal 69 amino acids of rat GlcNAc T-V (see Example 14 for description of cloning). The DNA encoding the remainder of GlcNAc T-V was fused to the human gamma-interferon signal sequence coding region, and there is a Gln residue derived from the gamma-interferon at the N-terminus of the soluble GlcNAc T-V. The ordinary skilled artisan can readily produce soluble GlcNAc T-V derivations using the sequences provided herein, taken with what is well known to the art. Spent medium from cells expressing the soluble rat GlcNAc T-V was chromatographed over a copper chelating column and over CM fast flow Sepharose to yield purified soluble GlcNAc T-V. Table 3 summarizes the results of soluble GlcNAc T-V purification as described in Example 15 herein. It was determined that there were protein bands of 95, 75 and 60 kDa which appeared to have enzymatic activity, although the 60 kDa band appeared to be less active. When EDTA (5 mM) is incorporated in the CM Sepharose column step, nearly all the protein is of about 95 kDa. Alternatively, a cocktail of protease inhibitors for maximizing the amount of 95 kDa protein can be added to the culture medium, removed for the copper chelation column, and provided again before the CM Sepharose cation exchange chromatography step. When no EDTA is used in the second column purification step, the predominant protein band revealed by SDS-PAGE is about 60 kDa, with minor bands at around 75 and 95 kDa. The N-terminal amino acid sequence analysis of the 60 kDa protein (purified by FPLC, gel filtration) is consistent with proteolytic cleavage between amino acids 283 and 284 of SEQ ID NO:16.

Detailed Description Text - DETX (63):

Gu et al., J. Biochem. (1993) 113:614-619, reported that GlcNAc T-V purified from the QC human lung cancer cell line exhibited a molecular weight of 73 kDa with an additional component of 60 kDa when SDS-PAGE was carried out under reducing conditions, and suggested that the 60 kDa component was a proteolytic product of the 73 kDa protein.

Detailed Description Text - DETX (229):

The CHO cDNA library was screened with a 5'342 bp rat GlcNAc T-V PCR amplimer which hybridizes 14 bp upstream from the ATG start codon and extends 328 nucleotides into the GlcNAc T-V coding region. Positive phage clones were then screened with a 3'320 bp rat GlcNAc T-V PCR amplimer which hybridizes 5 bp upstream from the TAG stop codon and extends into the 3' untranslated region. One putative CHO GlcNAc T-V clone hybridized to both the 5' and 3' rat GlcNAc T-V probes. Sequence analysis of the positive clone after second and third round plaque purification revealed the full length CHO GlcNAc T-V cDNA sequence (FIG. 11A-11F, SEQ ID NO:17)

Detailed Description Text - DETX (232):
Determination of Human GlcNAc T-V Sequence

Detailed Description Text - DETX (233):
The sequences for human GlcNAc T-V was readily determined using standard molecular biology procedures and the rat GlcNAc T-V sequence information provided herein.

Detailed Description Text - DETX (238):
The human GlcNAc T-V sequence upstream from the kidney PCR product was obtained by amplifying sequences in a commercially available human placenta cDNA library (Stratagene, #936203). Specific oligonucleotide primers from the human kidney PCR sequence were used as the 3' end antisense primers and a primer covering the T7 promoter sequence of the library cloning vector .lambda.ZAPII (Stratagene) was used as the 5' end sense primer. PCR was carried out using the following primers:

Detailed Description Text - DETX (243):
A comparison of the rat GlcNAc T-V sequence with the human sequence obtained by PCR of the human placenta cDNA library revealed that the human sequence lacked 14 bp from the ATG initiation codon. In order to obtain the extreme 5' end of the human GlcNAc T-V sequence, PCR was carried out using a sense primer designed to hybridize to sequences obtained from a portion of human GlcNAc T-V genomic DNA. The 5' primer sequence was as follows:

Detailed Description Text - DETX (247):
The human GlcNAc T-V sequence downstream from the approximately 1125 bp kidney PCR product was obtained by amplifying sequences in the human placenta cDNA library using a specific oligonucleotide primer from the human kidney PCR sequence as the 5' end sense primer and a primer covering the T3 promoter sequence of the library cloning vector .lambda.ZAPII (Stratagene) as the 3' end antisense primer. PCR was carried out using the following primers:

Detailed Description Text - DETX (255):
Using standard procedures and following the teachings of the cited patent, the cleavable signal sequence of human gamma-interferon was fused with the rat GlcNAc T-V at the sequence corresponding to amino acid number 70 of SEQ ID NO:16. This chimera has replaced the GlcNAc T-V putative cytoplasmic domain (amino acids 1-13), transmembrane domain (amino acids 14-30) and a portion of the stem region (amino acids 31-69) with a fragment coding for the 23 amino acid signal peptide and first amino acid of mature human gamma-interferon. The resulting fusion gene product is cleaved to yield secretable GlcNAc T-V containing one amino acid from the gamma-interferon (Gln) at the new NH.sub.2 -terminus.

Detailed Description Text - DETX (269):
The secreted rat GlcNAc T-V expression vector, clone D from above, was transfected into CHO dhfr.sup.- cells by the calcium phosphate precipitation method (Graham and van der Eb, Virology (1973) 52:456-467) modified as described by Wigler et al. (Cell (1978) 41:725-731) and Lewis et al. (Somatic Cell Genetics (1980) 6:333-347). Following selection by growth in media containing 5% dialyzed FBS (Irvine Scientific), pools and clones of stably transfected CHO dhfr.sup.- cells were obtained. Cell conditioned media from the transfected CHO dhfr.sup.- cell lines were collected and analyzed by the radionucleotide assay as described in Part B. The CHO dhfr.sup.- cell line which produced the highest amount of active soluble GlcNAc T-V as determined by the radiochemical assay (709 pmol/mg.times.hr) was used to seed a spinner cell culture flask. The cells were propagated in suspension cell culture and then

used to seed roller bottles at an initial seeding density of 2.5.times.10.sup.7 cells in 200 ml of a 50/50 mixture of DMEM and F-12 media (Gibco) supplemented with 5% dialyzed FBS, 1.times. non-essential amino acids (Gibco) and 2 mM L-glutamine (Gibco). After three days the roller bottles were shifted to 200 ml of serum-free medium. Harvests were collected at 6-day intervals with new serum-free medium added after each harvest. In total, 62 liters of conditioned medium were harvested and concentrated to 2.4 liters by cross-flow ultrafiltration through Mini Sartocon polysulfone modules (Sartorius Corporation, Bohemia, N.Y.) then stored at -80.degree. C. prior to purification. Radionucleotide assays were carried out as described in Example 3 to analyze the GlcNAc T-V activity in the concentrated conditioned medium; the results demonstrated that approximately 26 units of total enzyme activity were produced.

Claims Text - CLTX (3):
3. The DNA molecule of claim 2 wherein said nucleotide sequence encodes human GlcNAc T-V.

Claims Text - CLTX (17):
17. The recombinant cell of claim 15 wherein said nucleotide sequence encodes human GlcNAc T-V.

Claims Text - CLTX (28):
25. The method of claim 24 wherein said nucleotide sequence encodes human GlcNAc T-V.

Other Reference Publication - OREF (1):
Saito et al. (1994) "cDNA Cloning and Chromosomal Mapping of Human N-acetylglucosaminyltransferase V", Biochem. & Biophys. Res, Comm. 198:318-327.

Other Reference Publication - OREF (2):
Gu et al. (1993) "Purification and Characterization of UDP-N-Acetylglucosamine: .alpha.-6-D-Maanoside .beta.1-6N-Acetylglucosaminyltransferase (N-Acetylglucosaminyltransferase V) from a Human Lung Cancer Cell Line", J. Biochem. 113:614-619.

US-PAT-NO: 5707846

DOCUMENT-IDENTIFIER: US 5707846 A

TITLE: N-acetylglucosaminyl transferase gene coding therefor and process for production thereof

DATE-ISSUED: January 13, 1998

INVENTOR-INFORMATION:

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APPL-NO: 08/ 405230

DATE FILED: March 16, 1995

PARENT-CASE:

This application is a divisional of application Ser. No. 08/110,736, filed Aug. 23, 1993.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	4-245950	August 24, 1992
JP	5-237118	August 6, 1993

US-CL-CURRENT: 435/193

ABSTRACT:

A .beta.1,6-N-acetylglucosaminyl transferase having the following properties:

(1) Action: it transfers N-acetylglucosamine from UDP-N-acetylglucosamine to .alpha.-6-D-mannoside;

(2) Substrate specificity: it shows a reactivity of about 79% for GnGnF-bi-PA, about 125% for GnGnGn-tri-PA and about 66% for GnM-Pa, when taking a reactivity for GnGn-bi-PA as 100%;

(3) Optimum pH: 6.2 to 6.3;

(4) Inhibition, Activation and Stability: Mn.sup.2+ is not necessary for expression of activity, and the activity is not inhibited in the presence of 20 mM EDTA;

(5) Molecular weight: about 73,000 as determined by SDS-PAGE in the absence of reducing agent; and about 73,000 and about 60,000 as determined in the presence of a reducing agent;

(6) Km value: 133 .mu.M and 3.5 mM for acceptor GnGn-bi-PA and donor UDP-GlcNAc, respectively; and

(7) It includes the following peptide fragments:

(SEQ ID NO.1) Thr-Pro-Trp-Gly-Lys

(SEQ ID NO.2) Asn-Ile-Pro-Ser-Tyr-Val

(SEQ ID NO.3)
Val-Leu-Asp-Ser-Phe-Gly-Thr-Glu-Pro-Glu-Phe-Asn-His-Ala-Asn-Tyr-Ala

(SEQ ID NO.4) Asp-Leu-Gln-Phe-Leu-Leu

(SEQ ID NO.5) Asn-Thr-Asp-Phe-Phe-Ile-Gly,

and gene coding for said enzyme, and a process for production of the enzyme.

10 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Brief Summary Text - BSTX (11):
Among these six N-acetylglucosaminyl transferases, human and rabbit cDNA structures for GnT-I were clarified (Kumar, R., Yang, J., Larsen, R. C., and Stanley, P. Procc. Natl. Acad. Sci. USA, 87, 9948-9952 (1990) and Sarkev, M. Hull, E., Nishikawa, Y., Simpson, R. J., Noritz, R. L., Dunn, R., and Schachter, H., Proc. Natl. Acad. Sci. U.S.A 88 234-238 (1991).

Claims Text - CLTX (11):
4. The isolated .beta.1,6-N-acetylglucosaminyl transferase according to claim 3 wherein the modification is an addition, deletion, replacement with other nucleotides, or a combination thereof.

Claims Text - CLTX (12):
5. The .beta.1,6-N-acetylglucosaminyl transferase of claim 1, wherein the transferase is of human origin.

Claims Text - CLTX (14):
7. The .beta.1,6-N-acetylglucosaminyl transferase of claim 5, wherein the transferase is derived from human lung carcinoma cells.

Claims Text - CLTX (15):
8. The .beta.1,6-N-acetylglucosaminyl transferase of claim 7, wherein the transferase is derived from human lung carcinoma SBM331 (FERM BP-3967).

US-PAT-NO: 5624832

DOCUMENT-IDENTIFIER: US 5624832 A
See image for Certificate of Correction

TITLE: .beta.1 6 N-acetylglucosaminyltransferase, its acceptor molecule, leukosialin, and a method for cloning proteins having enzymatic activity

DATE-ISSUED: April 29, 1997

INVENTOR-INFORMATION:

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APPL-NO: 08/ 227455

DATE FILED: April 14, 1994

PARENT-CASE:

This application is a divisional of application Ser. No. 07/995,041, filed Oct. 1, 1992, now U.S. Pat. No. 5,360,733.

US-CL-CURRENT: 435/193, 435/252.3 , 435/254.11 , 435/320.1 , 435/325 , 435/348 , 435/358 , 435/361 , 536/23.2

ABSTRACT:

The present invention provides a novel .beta.1.fwdarw.6 N-acetylglucosaminyltransferase, which forms core 2 oligosaccharide structures in O-glycans, and a novel acceptor molecule, leukosialin, CD43, for core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase activity. The amino acid sequences and nucleic acid sequences encoding these molecules, as well as active fragments thereof, also are disclosed. A method for isolating nucleic acid sequences encoding proteins having enzymatic activity is disclosed, using CHO cells that support replication of plasmid vectors having a polyoma virus origin of replication. A method to obtain a suitable cell line that expresses an acceptor molecule also is disclosed.

9 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Brief Summary Text - BSTX (15):
The present invention generally relates to a novel purified human .beta.1.fwdarw.6 N-acetylglucosaminyltransferase. A cDNA sequence encoding a 428 amino acid protein having .beta.1.fwdarw.6 N-acetylglucosaminyltransferase activity also is provided. The purified human .beta.1.fwdarw.6 N-acetylglucosaminyltransferase, or an active fragment thereof, catalyzes the formation of critical branches in O-glycans.

Drawing Description Text - DRTX (10):
The present invention generally relates to a novel human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase. The invention further relates to a novel method of transient expression cloning in CHO cells that was used to isolate the cDNA sequence encoding human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase (C2GnT). The invention also relates to a novel human leukosialin, which is an acceptor molecule for core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase activity.

Drawing Description Text - DRTX (19):
An enzyme similar to the disclosed human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase has been purified from bovine tracheal epithelium (Ropp et al., J. Biol. Chem. 266:23863-23871 (1991), which is incorporated herein by reference. The apparent molecular weight of the bovine enzyme is .about.69 kDa. In comparison,, the predicted molecular weight of the polypeptide portion of core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase is .about.50 kDa. The deduced amino acid sequence of core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase reveals two to three potential N-glycosylation sites, suggesting N-glycosylation and O-glycosylation, or other post-translational modification, could account for the larger apparent size of the bovine enzyme.

Drawing Description Text - DRTX (26):
The present invention is directed to a method for transient expression cloning in CHO cells of cDNA sequences encoding proteins having enzymatic activity. Isolation of human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase is provided as an example of the disclosed method. However, the method can be used to obtain cDNA sequences encoding other proteins having enzymatic activity.

Drawing Description Text - DRTX (28):
As used herein, the terms "purified" and "isolated" mean that the molecule or compound is substantially free of contaminants normally associated with a native or natural environment. For example, a purified protein can be obtained from a number of methods. The naturally-occurring protein can be purified by any means known in the art, including, for example, by affinity purification with antibodies having specific reactivity with the protein. In this regard, anti-core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase antibodies can be used to substantially purify naturally-occurring core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase from human HL-60 promyelocytes.

Drawing Description Text - DRTX (31):
The present invention is further directed to active fragments of the human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase protein. As used herein, an active fragment refers to portions of the protein that substantially retain the glycosyltransferase activity of the intact core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase protein. One skilled in the art can readily identify active fragments of proteins such as core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase by comparing the activities of a selected fragment with the intact protein following the guidance set forth in the Examples below.

Drawing Description Text - DRTX (35):
Relatedly, the invention also provides nucleic acids encoding the human core 2 .beta.1.fwdarw.6 N-acetylglucosaminyltransferase protein and leukosialin protein described above. The nucleic acids can be in the form of DNA, RNA or cDNA, such as the novel C2GnT cDNA of 2105 base pairs identified in FIG. 5 (SEQ. ID. NO. 3) or the novel leukosialin cDNA identified in FIG. 2 (SEQ.

ID. No. 1), for example. Such nucleic acids can also be chemically synthesized by methods known in the art, including, for example, the use of an automated nucleic acid synthesizer.

Claims Text - CLTX (1):
1. An isolated nucleic acid molecule encoding a human protein having .beta.1.fwdarw.6-N-acetylglucosaminyltransferase activity only for a core 1-containing acceptor molecule.

Claims Text - CLTX (9):
9. An isolated nucleic acid molecule encoding the amino acid sequence shown as amino acids 38 to 428 in FIG. 5 (SEQ. ID. NO. 4), wherein said amino acid sequence is an active fragment of a human protein having .beta.1.fwdarw.6-N-acetylglucosaminyltransferase activity only for a core 1-containing acceptor molecule.

Other Reference Publication - OREF (14):
Kawashima, Hiroto, et al., "Purification and Characterization of UDP-GlcNAc:Gal.beta.1-4Glc (NAc) .beta.-1, 3-N-Acetylglucosaminyltransferase (Poly-N-acetyllactosamine Extension Enzyme) from Calf Serum." J. Biol. Chem. 268:27118-27126 (1993).

US-PAT-NO: 5501957

DOCUMENT-IDENTIFIER: US 5501957 A

TITLE: Method for measuring glycosyltransferase activity

DATE-ISSUED: March 26, 1996

INVENTOR-INFORMATION:

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APPL-NO: 08/ 293940

DATE FILED: August 22, 1994

PARENT-CASE:

This application is a continuation of Ser. No. 07/968,865, filed Oct. 30, 1992, now abandoned.

US-CL-CURRENT: 435/15, 435/14 , 435/4 , 435/810 , 435/97 , 436/92 , 436/94 , 514/1 , 514/23 , 514/54 , 536/1.11 , 536/29.11

ABSTRACT:

A method of assaying for glycosyltransferase activity in a sample. In a first step, a sample is reacted with a first sugar donor and an acceptor substrate to produce a transferase product. The first sugar donor and acceptor substrate are selected such that the sugar from the first sugar donor is capable of being transferred to the acceptor substrate in the presence of the glycosyltransferase to be assayed. In a second step, the transferase product is reacted with a second sugar donor having a sugar which is labelled with a labelling agent and an enzyme which is capable of transferring the sugar from the second sugar donor to the transferase product to produce a labelled transferase product and which has a higher affinity for the glycosyltransferase product compared to the affinity of the glycosyltransferase for the acceptor substrate. The labelling agent activity of the labelled transferase product or unreacted second sugar donor is assayed to determine transferase activity in the sample. A kit for assaying for glycosyltransferase activity in a sample is also described.

28 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

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Brief Summary Text - BSTX (7):

Changes in the activity of core 2 GlcNAc-T have also been associated with the Wiskott-Aldrich immunodeficiency syndrome (WAS). Increased core 2 GlcNAc-T activity is closely associated with activation of human T cells in vitro, via

the T cell receptor complex (Piller, F. et al, J. Biol. Chem. 263:15146, 1988). Furthermore, lymphocytes of patients with WAS show abnormal regulation of the enzyme (Higgins, L. A. J. Biol. Chem. 266:6280, 1991).

Brief Summary Text - BSTX (18):

The method of the invention may be used to assay for glucosaminyltransferases including the following: UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosaminyltransferase. The method of the invention may also be used to assay for glucosyltransferases.

Brief Summary Text - BSTX (22):

The kit may be used to assay for glucosaminyltransferases such as UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosaminyltransferase. The kit may also be used to assay for glucosyltransferases.

Claims Text - CLTX (12):

10. A method as claimed in claim 9 wherein the glucosaminyltransferase to be assayed is UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosamimyltransferase.

Claims Text - CLTX (24):

22. A kit as claimed in claim 21 wherein the glucosaminyltransferase to be assayed is UDP-GlcNac:Gal.beta.3GalNAc-R .beta.6-N-acetylglucosaminyltransferase; UDP-GlcNAc:GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:.alpha.3Man .beta.2-N-acetylglucosaminyltransferase I; UDP-GlcNAc:Gal .beta.4GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.3GalNAc-R .beta.3-N-acetylglucosaminyltransferase; UDP-GlcNAc: dolichol diphospho N-acetylglucosamine .beta.1-4 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-3GlcNAc-R .beta.1-3 N-acetylglucosaminyltransferase; UDP-GlcNAc:Gal.beta.1-4GlcNAc-R .beta.1-6 N-acetylglucosaminyltransferase; or UDP-GlcNAc:Gal.beta.1-4Glc .beta.-R .beta.1-3 N-acetylglucosaminyltransferase.